

Prevalence and etiology of subclinical mastitis in Awassi dairy ewes in southern Turkey

Yaşar ERGÜN^{1,*}, Özkan ASLANTAŞ², Gökhan DOĞRUER¹, Ekrem KİREÇCİ³,

Mustafa K. SARIBAY¹, Cafer T. ATEŞ⁴, Alev ÜLKÜ¹, Cemil DEMİR²

¹Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine,

Mustafa Kemal University, Hatay - TURKEY

²Department of Microbiology, Faculty of Veterinary Medicine, Mustafa Kemal University, Hatay - TURKEY

³Department of Microbiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum - TURKEY

⁴Department of Animal Husbandry, Faculty of Veterinary Medicine, Mustafa Kemal University, Hatay - TURKEY

Received: 18.03.2008

Abstract: In order to study the prevalence and etiology of subclinical mastitis a bacteriological survey on 16 Awassi dairy sheep flocks in southern Turkey was conducted. A total of 1458 milk samples from 729 Awassi ewes in mid-lactation were tested with the California mastitis test (CMT). Samples from 170 (11.7%) glands and 135 (18.5%) sheep had positive CMT results. Bacteria were isolated from 93 (6.4%) udder halves and 82 (11.2%) ewes. Positive CMT and bacteriological results were combined to define subclinical mastitis. The prevalence of subclinical mastitis and positive CMT samples among the different flocks ranged from 1.9% to 11.5% and 2.8% to 21.9% of the glands, and 3.8% to 19% and 5.7% to 31.3% of the ewes, respectively, with averages of 6.4% and 11.7% of the glands, and 11.2% and 18.5% of the ewes, respectively. Coagulase-negative staphylococci (CNS) were the most prevalent bacteria, representing 76.5% of the isolates. *Staphylococcus epidermidis* (35.7%) was the most prevalent species, followed by *Staphylococcus xylosum* (10.2%), *Staphylococcus saprophyticus* (10.2%), *Staphylococcus warneri* (9.2%), and *Staphylococcus intermedius* (7.1%). Antimicrobial susceptibility of 78 *Staphylococcus* isolates was evaluated in this study. The most effective antibiotics were cephalothin (97.4%), sulfamethoxazole-trimethoprim (97.4%), amoxicillin + clavulanic acid (97.4%), enrofloxacin (94.9%), gentamycin (92.3%), and erythromycin (84.6%). The relationship between CMT +1 score and the *Staphylococcus* spp. isolation rate was statistically significant ($P < 0.001$). It was concluded that subclinical mastitis is not highly prevalent and it does not pose a significant health problem for milking Awassi sheep flocks in southern Turkey.

Key words: Subclinical mastitis, Awassi ewe, prevalence, etiology, southern Turkey

Türkiye'nin güneyindeki İvesi sürülerinde subklinik mastitisin prevalans ve etiyolojisi

Özet: Bu çalışma, Türkiye'nin güney bölgelerinde yetiştirilen 16 İvesi sürüsünde subklinik mastitislerin prevalans ve etiyolojisinin belirlenmesi amacıyla yapıldı. Çalışma materyalini 729 ivesi koyununa ait 1458 süt örneği oluşturdu. Örnekler laktasyonun orta döneminde, California mastitis testi (CMT) uygulandıktan sonra alındı. Çalışma materyalini oluşturan koyunların 135'inde (% 18,5) ve meme yarımalarının 170'inde (% 11,7) CMT pozitif sonuç elde edildi. Bakteri izolasyonu 93 (% 6,4) meme lobundan ve 82 (% 11,2) koyundan gerçekleştirildi. Hem CMT sonucu pozitif hem de bakteri izolasyonu yapılan örnekler mastitis olarak değerlendirildi. Subklinik mastitis ve CMT pozitif örneklerin sürülerde meme

* E-mail: yasar@mku.edu.tr

yarımı bazında dağılımı sırasıyla, % 1,9 ile % 11,5 ve % 2,8 ile % 21,9 arasında; koyun bazında dağılım ise sırayla % 3,8 ile % 19,0 ve % 5,7 ile % 31,3 arasında gerçekleşti. Subklinik mastitis ve CMT pozitif örneklerde ortalama dağılımlar ise meme yarımı bazında sırayla, % 6,4 ile % 11,7 ve koyun bazında % 11,2 ile % 18,5 olarak belirlendi. Koagülaz negatif stafilocoklar (KNS) % 76,5 ile en sık izole edilen bakteri grubunu oluşturdu. En sık izole edilen bakteri *Staphylococcus epidermidis* (% 35,7) olarak belirlendi, bunu *Staphylococcus xylosus* (% 10,2), *Staphylococcus saprophyticus* (% 10,2), *Staphylococcus warneri* (% 9,2), ve *Staphylococcus intermedius* (% 7,1) izledi. Antibiyotik duyarlılık testi uygulanan 78 stafilocok izolatına en etkili antibiyotiklerin sırasıyla, cephalothin (% 97,4), sulfamethoxazole-trimethoprim (% 97,4), amoxicillin + clavulanic acid (% 97,4), enrofloxacin (% 94,9), gentamycin (% 92,3) ve erythromycin (% 84,6) olduğu belirlendi. CMT skoru +1 olan örneklerden stafilocok türlerinin izole edilmesi istatistik açıdan önemli bulundu ($P < 0,001$). Sonuç olarak, Türkiye'nin güney bölgesindeki İvesi sürülerinde subklinik mastitis oranının düşük olduğu ve önemli bir sürü problemi olmadığı belirlendi.

Anahtar sözcükler: Subklinik mastitis, İvesi koyunu, prevalans, etiyoloji, Türkiye

Introduction

Mastitis is a significant problem in dairy sheep flocks and leads to decreased milk production. Mastitis has importance for 3 perspectives: economic, hygienic, and legal (E.U. Directive 46/92, modified by Directive 71/94). The prevalence of subclinical sheep mastitis ranges between 7.05% and 92% and it occurs worldwide. The economic importance is particularly significant in the Mediterranean countries (1,2). Turkey is the second highest sheep milk producer among the European countries, with 790,000,000 L produced in 2005, and is one of the world's major sheep milk producers (3).

Coagulase-negative staphylococci (CNS) are the primary causative agents of subclinical mastitis (30%-95%) in dairy ewes (2,4-6). The most commonly isolated CNS species in persistent subclinical mastitis of sheep are *Staphylococcus epidermidis*, *Staphylococcus simulans*, *Staphylococcus chromogenes*, *Staphylococcus xylosus*, and *Staphylococcus haemolyticus* (6). The presence of different CNS species could be attributable to variation in mastitis control practices, such as the protocol and type of disinfectant used for teat dipping or dry-off treatments (5). The California mastitis test (CMT) is very useful, easy to perform, and low-cost tool for detecting subclinical mastitis in ewes. It should be carried out before milking to take into account somatic cell count (SCC) variations associated with milk fractions (7). CMT score is positively correlated with SCC, infection status, and the number of bacteria isolated in small ruminants (8,9). The negative predictive value of CMT is greater than its positive predictive value (4,10).

Subclinical mastitis affects the hygienic qualities and physicochemical properties of milk (11), which is of special concern to producers due to European regulation of the hygienic quality of sheep milk. Subclinical mastitis represents, therefore, an important risk to the competitiveness of the economic sectors related to sheep milk production. It is necessary to identify the microorganisms causing subclinical mastitis in each flock for establishing specific and efficient control methods because of the diversity of the agents (10,12-14).

The present study aimed to determine the prevalence of subclinical mastitis in Awassi ewes in southern Turkey, to identify the pathogens responsible, and to determine the effective antibiotics against the isolated microorganisms.

Materials and methods

Flocks and animals

The animal material consisted of 729 primiparous and multiparous native dairy Awassi ewes from 16 flocks in Hatay region in southern Turkey. All flocks were grazed, with some additional concentrate during spring, summer, and fall. Ewes were mostly housed during winter and fed wheat straw, barley grain, and wheat bran. All ewes lambed between January and March, and lambs were kept with their dams for 6-8 weeks. The average lactation period for most of the Awassi ewes was 150-180 days. Ewes selected for this study were apparently healthy, and free of clinical mastitis and any other palpable udder lesion. All ewes were in the mid lactation stage and were milked by hand. Milking hygiene, dry-sheep therapy, pre-

dipping or teat dipping procedures were not used in any of the 16 flocks we studied.

California mastitis test (CMT)

CMT was performed on all milk samples on the farms using the method described by Schalm et al. (15). According to visible reactions the results were classified into 5 scores: (0) = negative, (\pm) = trace, (+1) = weak positive, (+2) = distinct positive, and (+3) = strong positive.

Sample collection

Sheep were sampled before the morning or evening milkings and only CMT positive udder halves were sampled. 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 mL of milk was taken in a sterile tube for bacteriological examinations. Samples were collected aseptically according to a standard procedure (16) and transferred to the laboratory within 1-3 h in a 4-8 °C cooler.

Bacteriology

Bacteriological analysis was performed according to accepted standards (17). From each milk sample, 100 μ L was inoculated on blood-agar plates (Bacto-Agar, Difco Laboratory) containing defibrinated sheep blood, on MacConkey plates, and on Sabouraud dextrose agar. All plates were incubated at 37 °C and examined for growth between 24 and 72 h. Bacterial species initially identified by their colony morphology and Gram staining. The staphylococcal isolates were identified as *Staphylococcus aureus* and coagulase negative staphylococci (CNS) by using catalase, tube coagulase, and fermentation test for production of acid from glucose, mannitol, and maltose. CNS strains were identified to species level by using a microtube identification system (API STAPH-IDENT, bioMerieux S.A., Marcy-l'Etoile, France). *Escherichia coli* was identified by catalase, oxidase, growth on MacConkey agar, IMVIC test, and metallic sheen on EMB agar. *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and other streptococci were identified by CAMP test, esculin hydrolysis, hemolysis, and no growth on MacConkey agar. *Bacillus* spp. were identified by wide hemolysis zone and endospore formation; *Corynebacterium* spp.

were identified by hemolysis, nitrate reduction, and urease; and *Pseudomonas* spp. were identified by oxidase and growth on MacConkey agar. *Micrococcus* spp. were identified by cell arrangement and oxidation/fermentation test.

Antibiotic susceptibility test

Susceptibility testing was performed and evaluated according to the recommendations of CLSI (18). All isolates from dairy sheep with subclinical mastitis (n = 78) were tested with 9 different antibiotics: cephalothin (KF) (30 μ g), trimethoprim-sulfamethoxazole (STX) (1.25-23.75 μ g), amoxicillin-clavulanic acid (AMC) (20 μ g-10 μ g), enrofloxacin (ENR) (5 μ g), gentamycin (GM) (10 μ g), erythromycin (E) (5 μ g), tetracycline (TE) (10 μ g), ampicillin (AM) (10 μ g), and penicillin (P) (10 IU), all of which are widely used in veterinary practice in Turkey. In addition, oxacillin (OXA 1 μ g) was used to determine methicillin resistance of staphylococci. Antimicrobial susceptibility discs were obtained from Oxoid (Oxoid Limited England), except for ENR (Bayer Türk Kimya San., İstanbul, Turkey).

Definition of subclinical mastitis

Positive CMT and positive microorganism growth samples were together considered as subclinical mastitis.

Statistical analysis

Statistically significant associations were determined by the chi-square test. All statistical analyses were performed using SPSS v.13.0 software.

Results

Prevalence of subclinical mastitis

The prevalence of subclinical mastitis and positive CMT results in the 16 flocks sampled are shown in the Figure.

Subclinical mastitis and positive CMT results ranged from 3.8% to 19.0% and 5.7% to 31.3%, respectively, for ewes, and from 1.9% to 11.5% and 2.8% to 21.9%, respectively, for udder halves. The average prevalence of subclinical mastitis and positive CMT results detected in this study were 11.2% and 18.5%, respectively, for ewes, and 6.4%, and 11.7%, respectively, for glands. Most of the mammary

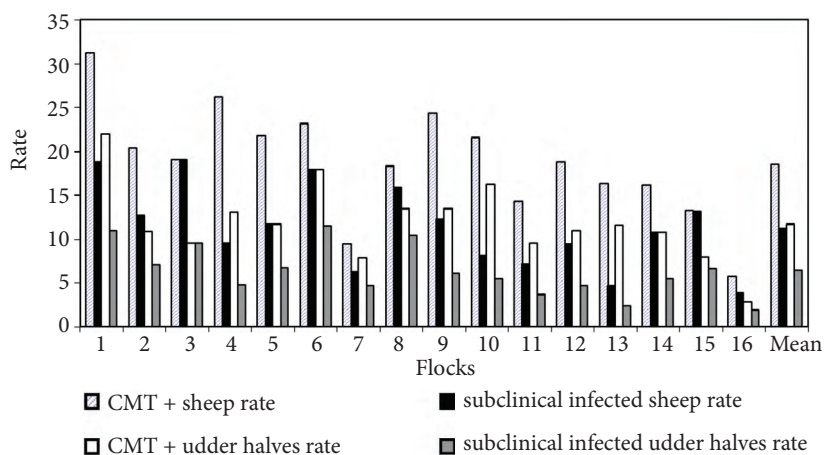


Figure. Prevalence of positive CMT results and subclinical mastitis rates, with respect to ewes and mammary glands, in 16 dairy sheep flocks in southern Turkey.

infections (86.6%) were unilateral and only 13.4% of the animals had bilateral infection ($P < 0.001$). The prevalence of subclinical mastitis in ewes in the first lactation and in ewes with 2 or more lactations was 4.1% and 7.4%, respectively, for glands and these results were statistically significant ($P < 0.05$). The infected right halves and positive CMT gland rates were 50.5% and 52.9%, respectively, and the infected left halves and positive CMT glands rates were 49.5% and 47.1%, respectively. The differences between left and right halves infection and positive CMT results rates were not significant. The lowest prevalence of subclinical mastitis was observed in primiparous ewes (8.1%), followed by ewes with 2 or more lactations (12.6%). The sensitivity of positive CMT results in this study was 60.7%.

Relationship between CMT positive results and bacteriological isolations

CMT and bacteriological results were compared in all flocks, and the results are shown in the Figure. Microorganism isolation rates were 57.3%, 51.3%, and 51.3% in CMT +1, +2, and +3 samples, respectively. CMT +1 had the highest diagnostic accuracy (57.3%).

Bacterial isolates

The number of bacterial species isolated from mammary glands and their distribution are shown in Table 1. CNS were the most prevalent bacteria. The most prevalent species was *S. epidermidis*,

representing 35.7% of the isolates, followed by *S. saprophyticus* (10.2%), *S. xylosum* (10.2%), *S. warneri* (9.2%), and *S. intermedius* (7.1%) (Table 1). Other CNS species isolated with a low frequency were *S. capitis* (2.0%), *S. cohnii* (1.0%), and *S. simulans* (1.0%). *S. aureus* represented 3.1% of the strains and it was isolated from 3 flocks. Streptococci were the second most prevalent bacterial group isolated from the samples. *S. uberis* and *S. dysgalactiae* represented 6.1% and 2.0%, respectively, and *S. agalactiae* represented 2.0% of the strains identified as *Streptococcus* sp. although it was isolated only from 1 flock. Other bacteria isolated at a low frequency were *Pseudomonas* spp., *Escherichia coli*, *Bacillus* spp., *Micrococcus* spp., and *Corynebacterium* spp., representing 2.0%, 2.0%, 2.0%, 1.0%, and 1.0% of isolated strains, respectively.

In general, there was diversity in the species isolated from each flock; ≥ 3 different microorganisms were isolated from 87.5% of the flocks. *S. epidermidis*, the most widely distributed species, was isolated from 81.3% of the flocks. *S. xylosum* and *S. saprophyticus* were isolated from 43.8% of the flocks. Distribution of other CNS species is shown in Table 1. *S. aureus* was isolated from 3 flocks (18.8%) and *S. warneri* from 5 flocks. It is noted that of the 75 CNS species isolated, 44 of them were isolated from CMT +1 samples and this result was statistically significant ($P < 0.001$).

Table 1. Bacterial isolation rate and microorganism distribution according to CMT results among dairy sheep with subclinical mastitis in southern Turkey.

Microorganism	Isolate number	% total isolate	% flocks	Microorganisms number isolated from		
				CMT +1 samples	CMT +2 samples	CMT +3 samples
Coagulase negative staphylococci	75	76.5	100	44	16	15
<i>S. epidermidis</i>	35	35.7	81.3	20	10	5
<i>S. xylosus</i>	10	10.2	43.8	7	2	1
<i>S. saprophyticus</i>	10	10.2	43.8	5	1	4
<i>S. warneri</i>	9	9.2	31.3	5	2	2
<i>S. intermedius</i>	7	7.1	31.3	4	1	2
<i>S. capitis</i>	2	2	12.5	1	0	1
<i>S. cohnii</i>	1	1	6.3	1	0	0
<i>S. simulans</i>	1	1	6.3	1	0	0
<i>S. aureus</i>	3	3.1	18.8	2	1	0
Streptococci	12	12.2	43.8	6	2	4
<i>S. agalactiae</i>	2	2	6.3	0	0	2
<i>S. uberis</i>	6	6.1	25	4	1	1
<i>S. dysgalactiae</i>	2	2	12.5	2	0	0
α -hemolytic streptococcus	1	1	6.3	0	0	1
β -hemolytic streptococcus	1	1	6.3	0	1	0
Other microorganisms	8	8.2	31.3	6	0	2
<i>E. coli</i>	2	2	12.5	1	0	1
<i>Pseudomonas</i> spp.	2	2	6.3	2	0	0
<i>Bacillus</i> spp.	2	2	6.3	2	0	0
<i>Micrococcus</i> spp.	1	1	6.3	0	0	1
<i>Corynebacterium</i> spp.	1	1	6.3	1	0	0
Total	98			58	19	21
Mix isolates numbers	5			3	0	2
CMT positive samples	170			96	37	37

Table 2. Antibiotic susceptibility results of 78 staphylococcus species in dairy ewes in southern Turkey.

	KF	AMC	TS	ENR	GM	E	TE	AM	P
Susceptible	97.4%	96.2%	97.4%	94.9%	82.1%	75.6%	59.0%	50.0%	30.8%
Intermediate	-	1.3%	-	-	10.3%	9.0%	16.7%	7.7%	12.8%
Total Susceptible	97.4%	97.4%	97.4%	94.9%	92.3%	84.6%	75.6%	57.7%	43.6%
Resistant	2.6%	2.6%	2.6%	5.1%	7.7%	15.4%	24.4%	42.3%	56.4%

Antibiotic susceptibility results of the *Staphylococcus* isolates

Antimicrobial susceptibility of 78 *Staphylococcus* isolates was evaluated using 9 antibiotics or antibiotic combinations (KF, TS, AMC, ENR, GM, E, TE, AM, and P) and is shown in Table 2. The most effective antibiotics were KF (97.4%), TS (97.4%), AMC (97.4%), ENR (94.9%), GM (92.3%), and E (84.6%). Antibiotic resistance to P, AM, and TE was 56.4%, 42.3%, and 24.4%, respectively. No methicillin resistance was determined.

Discussion

This study was conducted to determine the prevalence of subclinical mastitis in Awassi milking sheep in southern Turkey and the etiology of the disease. The prevalence of subclinical mastitis in this study was recorded as 11.2% for sheep and 6.4% for glands. These infection rates are relatively low compared to those reported in other European, Eastern and Middle Eastern countries, such as Jordan 24.8% (19), Iran 39%, (20), Italy 17.5%, (21), Spain 34.6%, (2), Portugal 70%-92%, (22), Greece 17%-34%, (4,23), England 12% (24), and Israel 55% (25). Lower prevalence rates of subclinical mastitis have been reported in Austria 9%, (26) and Turkey 7.05% (27). The highest subclinical mastitis prevalence rates have been reported (22) in crossbred and purebred Awassi ewes (92% and 70%, respectively) in Portugal. Similar results have been reported by Fthenakis (28) during the second phase of lactation at an incidence of 11.2% in southern Greece. In the present study the prevalence of subclinical mastitis was lower (7.4%) in primiparous ewes than in ewes with 2 or more lactations (13.4%). Similar results were reported by Las Heras et al. (2). Positive CMT results as a sheep-side test are considered a useful indirect method for detecting subclinical mastitis. The sensitivity of the positive CMT results in the present study was 60.7%. CMT +1 score can be recommended as a threshold value for detecting subclinical mastitis, as reported by Las Heras et al. (2). Our data indicated that CMT should still be considered an effective screening test for subclinical mastitis in ewes. Similar results were reported for CMT +1 (28), and the sensitivity and specificity of CMT +1 scores were described as the

best CMT score for indicating subclinical mastitis in sheep and, therefore, a CMT +1 score can be recommended as the threshold value of a useful diagnostic farm-based test (2). We conclude that CMT is useful for classifying milk according to SCC, particularly when a simplified grid of interpretation is used; the efficiency ranges from 87% to 92% for scores (0) and (\pm), on the one hand and (+1), (+2), and (+3) on the other (2,9,29). Staphylococci were the most frequently isolated and most widespread microorganisms in the sample and they were present in all 16 flocks. In total, 9 *Staphylococcus* species were isolated and *S. epidermidis* was the most prevalent and widespread (81.3%) among the flocks (Table 1). This result is in agreement with previously published results (10,30). *S. xylosum*, *S. saprophyticus*, *S. warneri*, and *S. intermedius* were the other CNS species that followed *S. epidermidis* in importance and they were also widely distributed. Streptococci with a rate of 12.2% were the second most isolated bacterial group among the flocks. Streptococci were isolated in 43.8% of the flocks. The prevalence of corynebacteria in this study was low and similar to that reported by De la Cruz et al. (13) (0.5%) and by Marco (10) (2%).

As a result, the prevalence of subclinical mastitis observed in the present study was not higher than that of previous reports and the disease is not associated with a noticeable health threat for Awassi sheep flocks in southern Turkey. CMT +1 scores were the best CMT indicator of subclinical mastitis in the sheep. The relationship between CMT +1 score and isolation of *Staphylococcus* spp. was statistically significant ($P < 0.001$). Antibiotic resistance to some commonly used antibiotics (P, AM, and TE) was observed. It is recommended that KF, TS, AMC, ENR, and GM be used in the treatment of subclinical mastitis in sheep in this region.

In conclusion, milking hygiene, dry-sheep therapy, and pre-dipping or teat dipping procedures were not used in any of the 16 flocks we studied, and the prevalence of subclinical mastitis was lower than that of previous reports. The low prevalence of subclinical mastitis we observed in the present study may be due to the genetic factors and anatomical structure of Awassi sheep. Further studies should be conducted to determine the underlying nature of the Awassi sheep's resistance to mastitis.

References

1. Dario, C., Laudadio, V., Corsalini, T., Bufano, G., Buonavoglia, C.: Subclinical mastitis in sheep: occurrence, etiology and milk production in different genetic types. *Agr. Med.*, 1996; 126: 320-325.
2. Las Heras, A., Domínguez, L., Fernández-Garayzábal, J.F.: Prevalence and aetiology of subclinical mastitis in dairy ewes of the Madrid region. *Small Ruminant Res.*, 1999; 32: 21-29.
3. Turkish Statistical Institute, Access: http://tuik.gov.tr/PreIstatistikTablo.do?istab_id=684 access date: 24th November 2009.
4. Bergonier, D., Berthelot, X.: New advances in epizootiology and control of ewe mastitis. *Livest. Prod. Sci.*, 2003; 79: 1-16.
5. Contreras, A., Luengo, C., Sánchez, A., Corrales, J.C.: The role of intramammary pathogens in dairy goats. *Livest. Prod. Sci.*, 2003; 79: 273-283.
6. Contreras, A., Sierra, D., Sánchez, A., Corrales, J.C., Marco, J.C., Paape, M.J., Gonzalo, C.: Mastitis in small ruminants. *Small Ruminant Res.*, 2007; 68: 145-153.
7. Peris, C., Molina, P., Fernandez, N., Rodriguez, M., Torres, A.: Variation in somatic cell count, California mastitis test, and electrical conductivity among various fractions of ewe's milk. *J. Dairy Sci.*, 1991; 74: 1553-1560.
8. Contreras, A., Sierra, D., Correlas, J.C., Sanchez, A., Marco, J.: Physiological threshold of somatic-cell count and California mastitis test for diagnosis of caprine subclinical mastitis. *Small Ruminant Res.*, 1996; 21: 259-264.
9. McDougall, S., Murdough, P., Pankey, W., Delaney, C., Barlow, J., Scruton, D.: Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. *Small Ruminant Res.*, 2001; 40: 245-254.
10. Marco, J.C.: Mastitis in Latxa ewes: epidemiology, diagnostic and control. Ph.D. Thesis, University of Zaragoza, Spain, 1994.
11. Hamed, A.I., Abou-Zeid, N.A., Kebary, K.M.K., Radwan, A.A.: Physical and chemical properties of subclinical mastitic sheep's and goat's milk. *Egypt. J. Dairy Sci.*, 1993; 21: 133-149. (Ph. D. Thesis in Spanish, with an abstract in English).
12. González-Rodríguez, M.C., Gonzalo, C., San Primitivo, F., Cármenes, P.: Relationship between somatic cell count and intramammary infection of the half udder in dairy ewes. *J. Dairy Sci.*, 1995; 78: 2753-2759.
13. de la Cruz, M., Serrano, E., Montoro, V., Marco, J., Romeo, M., Baselga, R., Albizu, I., Amorena, B.: Etiology and prevalence of subclinical mastitis in the Manchega sheep at mid-late lactation. *Small Ruminant Res.*, 1994; 14: 175-180.
14. Lafi, S.Q., al-Majali, A.M., Rousan, M.D., Alawneh, J.M.: Epidemiological studies of clinical and subclinical ovine mastitis in Awassi sheep in northern Jordan. *Prev. Vet. Med.*, 1998; 33: 171-181.
15. Schalm, O.W., Carrol, E.J., Jain, N.C.: *Bovine Mastitis*. Lea and Febiger, Philadelphia, USA. 1971: 360.
16. International Dairy Federation, Milk and Milk Products: Guidance on Sampling. International Dairy Federation, Standard 50B, Brussels, Belgium. 1985.
17. Hogan, J.S., Gonzalez, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W., Smith, K.L.: *Laboratory Handbook on Bovine Mastitis*. rev. ed. NMC Inc., Madison, WI; 1999.
18. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement (M100-S16); Wayne PA, 2006.
19. Al-Majali, A.M., Jawabreh, S.: Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Ruminant Res.*, 2003; 47: 243-248.
20. Batavani, R.A., Mortaz, E., Falahian, K., Dawoodi, M.A.: Study on frequency, etiology and some enzymatic activities of subclinical ovine mastitis in Urmia, Iran. *Small Ruminant Res.*, 2003; 50: 45-50.
21. Albenzio, M., Taibi, L., Muscio, A., Sevi, A.: Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of ewe milk. *Small Ruminant Res.*, 2002; 43: 219-226.
22. Quiroga, M.C., Marcelion, P.P., Espadameria, E.M., Vilela, C.L.: Survey of mastitis in sheep: a preliminary study. *Vet. Tecnica*, 1997; 7: 52-55.
23. Fthenakis, G.C.: Prevalence and aetiology of subclinical mastitis in ewes of Southern Greece. *Small Ruminant Res.*, 1994; 13: 293-300.
24. Watkins, G.H., Burriel, A.R., Jones, J.E.T.: A field investigation of subclinical mastitis in sheep in southern England. *Br. Vet. J.*, 1991; 147: 413-420.
25. Bor, A., Winkler, M., Gootwine, E.: Non-clinical intramammary infection in lactating ewes and its association with clinical mastitis. *Br. Vet. J.*, 1989; 145: 178-184.
26. Schoeder, G., Baumgartner, W., Pernthaner, A.: Variation of somatic cell counts in sheep and goats milk during the lactation period. In: *Proc. 5th Symp. Machine Milking of Small Ruminants*. Budapest, Hungary. 1993; 99-104.
27. Baysal, T., Kenar, B.: Isolation and identification of aerobic microorganisms from clinical and subclinical mastitis cases in Konya and its vicinity. *Etlik Vet. Mikrobiyol. Derg.*, 1989; 6: 55-66. (article in Turkish, with an abstract in English)
28. Fthenakis, G.C.: California mastitis test and Whiteside test in diagnosis of subclinical mastitis of dairy ewes. *Small Ruminant Res.*, 1995; 16: 271-276.
29. González-Rodríguez, M.C., Cármenes, P.: Evaluation of the California mastitis test as a discriminant method to detect subclinical mastitis in ewes. *Small Ruminant Res.*, 1996; 21: 245-250.
30. Burriel, A.R.: Isolation of coagulase-negative staphylococci from the milk and environment of sheep. *J. Dairy Res.*, 1998; 65: 139-142.