

Research Article

Study on the Prevalence of Subclinical Mastitis and the related Bacterial Flora in the Raw Milk of Primiparous Indigenous Greek Goats

Tzora Athina^{*1}, Voidarou Chrissa¹, Giannenas Ilias², Tsinas Anastasios¹, Skoufos Ioannis¹

¹Department of Agriculture Technology, School of Agriculture Technology, Food Technology and Nutrition, Division of Animal Production, TEI of Epirus, Arta, Greece

²Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

*Corresponding author: Dr. Athina Tzora, University: Dept Of Agriculture technology School of agriculture Technology, Division of Animal Production, TEI of Epirus, Arta, Address: TEI Of Epirus, Arta, 47100, Greece, Email: Tzora@teiep.gr; Tel: +302681050202

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Abstract

The objective of this study was to investigate the occurrence, prevalence and etiology of subclinical mastitis in primiparous dairy goats of indigenous Greek breeds. A total number of 340 healthy primiparous goats were used. Milk samples were taken directly from both udder cistern from individual goats and were subjected to bacteriological analysis. Bacterial growth was observed in 34.57% of total milk samples. From the latter figure 51% concerned only the half - udder that was affected. Bacterial isolates were identified on the basis of colonial morphology, Gram staining and automated phenotypic identification system Vitek 2 (bioMérieux). The highest prevalence was observed on Coagulase Negative Staphylococci (CNS) 41.07% followed by *Staphylococcus aureus* 23.21% and Enterobacteriaceae 3.57%. The rest isolates were identified as environmental originated. A positive mastitis test reaction (CMT) was revealed in 46.91% of samples. Pathogenic bacteria were isolated from samples which were negative to CMT corresponding to a 28.5% percentage of the total bacterial positive samples. It is revealed that the CNS is the prevalent etiological agent of the subclinical mastitis in primiparous goats.

Keywords: Subclinical Mastitis; Bacterial Flora; Raw Milk; Primiparous Goats; Coagulase Negative Staphylococci

Abbreviations

CNS: Coagulase Negative Staphylococci ;

CMT: California Mastitis Test

Introduction

Goat breeding is an ancient activity of the Greek rural areas, well documented since antiquity. Greek indigenous breeds show an exceptional adaptation to the steep mountainous terrain as well as to the intense anaglyph of the islands' terrain [1]. This adaptability of the goat is much stronger than the one of the sheep and this is the main reason that these animals thrive in such environments. The local communities depend on the production of dairy products originating from these animals. Different kinds of cheese and yoghurt are manufactured in an artisan way in the farms and are sold in the local markets. Due to the current fiscal difficulties of Greece and the high unemployment rate in the urban areas, young farmers are opted for goat breeding, attracted by the lucrative perspectives of goat dairy products; goat dairying is currently one of the most rapidly expanding areas of agriculture. In that context the research interest is focused in the productivity of these animals as well as in the public health risk assessment. The herd management remains traditional and thus goat production can be regarded as low input system far from industrialized standards. The animals' feeding is based on grazing in the semi-mountainous areas and goats are fed concentrated feeds only during parturition and for the first period of lactation [2]. Indigenous breeds, due to their high level of adaptation to harsh ecosystems of low input systems, shape a robustness to health problems, however, mastitis and especially subclinical mastitis could be a threat with high prevalence [3].

Subclinical mastitis is a serious condition directly related to clinical mastitis, decreased milk production and related with public health issues. Subclinical mastitis in goats is common and in general the prevalence of subclinical mastitis is reported to a range from 9% to 50% [3,4]. Various tests have been proposed for the diagnosis of subclinical mastitis in goats e.g. the California Mastitis Test (CMT) or the measurement of activity of certain enzymes in the milk e.g. lactic dehydrogenase (LDH), alkaline phosphatase (AP) and others [5-11]. Somatic cells show large variations in goats' milk (unlike ovine or bovine milk) and hence interpretation of such results must be cautious [12]. The same remark is valid for the CMT results because they are correlated to somatic cells content of the milk [11]. There is not yet a consensus on the number of somatic cells per ml of goats' milk above which a reliable diagnosis of subclinical mastitis can be set. On the other hand the biochemical tests were significantly influenced by the week of lactation and parity, stage of lactation and parity and also demand special equipment which is very expensive [13]. These characteristics render them unsuitable for routine clinical use. Considering the microbiology of milk originating from udders affected by subclinical mastitis, the significance of such indicators is beyond questioning but equally important is the isolation of specific pathogens because it provides the possibility of epizootic evaluation, as well as public health risk assessment. Another factor that has been rather overlooked by many researchers is the prevalence of subclinical mastitis caused by saprophytes, opportunistic pathogens and other pathogens isolated rarely, such as *Klebsiella pneumonia*, *Proteus* spp.,

Bacillus spp., *Actinobacter* spp., *Micrococcus* spp. [14-18] These microorganisms being omnipresent may be the cause of infections in certain cases.

The aim of this study was to investigate the prevalence of subclinical mastitis in primiparous goats of indigenous Greek breeds with respect to pathogens, as well as to saprophytic microorganisms. While mastitis is a significant disease of small ruminants, less research has been done with dairy goats. Evaluation of the relation between subclinical mastitis and microbiology of the milk emerges a multitude of factors, such as the breed, parity, stage of lactation, environment, nutritional status, health of the animal and udder localization (left or right) are among them.

Material and Methods

Milk sampling

Seven herds of goats from the mountainous region of Epirus (Hellas) were randomly selected and characterized as A to G herds. From each herd a number of primiparous goats in the mid of the milking period were selected as A(n=40), B(n=40), C(n=60), D(n=40), E(n=64), F(n=40) and G(n=40) in total 324 animals and after discarding the first three streams of milk from each udder, a sample of 10-15 ml of secretion were carefully collected into a sterile container. Standard methods for collection of secretion samples were followed (Fthenakis 1994). All animals from herds A and F belonged to the Skopelos breed (production standards: 250 litter of milk per milking period in primiparous goats) and all the rest were indigenous dairy goats *Capra prisca* (production standard: 150 litter of milk per milking period in primiparous goats). Prior to sampling and testing all animals were thoroughly examined to ensure the absence of any clinical symptoms as well as the health of the udders, with special attention to their mammary glands and teats [19]. Disinfection was carried out using povidone iodine scrub solution on the teat apex and the lower (1 cm) part of the teat skin [20]. Samples were delivered to the laboratory in a cool box at less than 4°C within 8 h of collection and tested immediately upon arrival. Milk samples were analysed for fat, protein, lactose and total solids by using an infrared method MilkoScan 4000 (FOSS Electric, Integrated Milk Testing™, Denmark). Milk acidity was measured by a portable pH meter BT-600 (BOECO, Germany).

Bacteriological analysis and measurement of Somatic Cell Count (SCC)

Milk samples were cultured according to Standard reference methods [19,21]. The first decimal dilution (10^{-1}) in Ringer's solution was followed by further dilution up to 10^{-4} . After the samples were seeded in 5% sheep blood Columbia agar and incubated aerobically at 37 ± 1 °C for up to 48-72 h. Throughout this study, all bacteria isolated were identified by using conventional techniques (Barrow and Feltham 1993). Bacteriological tests were performed by means of standard methods of isolation and identification of individual species of bacteria according to ISO requirements. API-tests (bioMerieux) and Vitek 2 Identification System (bioM-

erieux) were used for biochemical determination.

The California Mastitis Test (CMT) was performed in all secretion samples, as described for ewes' milk [22]. The CMT scores were classified as negative (-), traces (\pm) or positive (+, ++, +++, +++++). Any score (+) or over was regarded as indicative of subclinical mastitis.

Statistical analysis

Pearson's chi square test and Spearman's rank test were used to evaluate statistically differences and correlations ($p < 0.05$) while OR values with their respective confidence intervals (95%) were calculated were appropriate.

Results and Discussion

The specific aim of this work was to investigate the occurrence, prevalence and etiology of subclinical mastitis in primiparous dairy goats of indigenous Greek breeds. Table 1 shows the CMT scores in every herd. The prevalence of CMT positive scores was 64.19% (208/324 animals). It has been well documented by various researchers that somatic cells count in goats' milk undergoes large variations in contrast to cows' milk and the relevance of SCS as a predictor of parameters of udder health and the susceptibility against mastitis is still untested [23,24]. The source of these variations can be attributed to many factors of infectious and non-infectious origin. Among the latter, parity, nutritional status, the season and condition of the pastures, the experience of the personnel of the farm, the breed and others are concerned [25]. In the experimental design of the present study effort was made to restrict those factors as much as possible by selecting herds from the same region and performing milk sampling during the same season. All primiparous goats were grazing in similar semi- mountainous pastures and were kept under similar conditions of management. However, the variation of CMT score as an indicator of somatic cells was intense and significant among the herds ($\chi^2=31.087$, 6df, $p < 0.001$) when CMT classes were merged and reclassified (0/+ scores one group and +/++/+++ /++++ scores the other group). This difference originated from the herds of the local indigenous dairy goats ($\chi^2=30.00$, 6df, $p < 0.001$) and not from those of the herds of the Skopelos breed (same classification as previously).

Table 1. CMT scores in seven goat herds of indigenous Greek breeds.

CMT	Herd A (n=40)	Herd B (n=40)	Herd C (n=60)	Herd D (n=40)	Herd E (n=64)	Herd F (n=40)	Herd G (n=40)
0	24	40	20	0	8	12	4
\pm	ND	ND	ND	ND	4	4	ND
+	16	ND	12	8	4	ND	16
++	ND	ND	8	4	16	16	20
+++	ND	ND	8	12	16	4	ND
++++	ND	ND	12	16	16	4	ND

This finding suggests that both breed and herd management interact to the CMT scores. Exactly 50% of the Skopelos goats

scored positively to CMT, while 68.85% of the local goats had a positive CMT score (OR=2.21, CI 95%: 0.788-6.17).

Table 2 shows the correlation of CMT scores to bacteriologically positive udders. From the 116 animals with (-) or (\pm) CMT score, 44 had microbiologically positive milk samples (37.93%), while from the 208 animals with CMT positive score, 116 had positive microbiology in their milk (55.76%). These findings are in accordance with the suggestion of other researchers that the CMT scores in goats' milk should be interpreted very cautiously because of its low predictive value in that species [26,27]. In the present study there was no statistical correlation between the CMT score and the number of animals with positive udder microbiology among the different herds. In ewes isolation of pathogens from udders that scored negative in CMT occurs in the early stages of infection [28]. A similar finding has not been reported in goats but the results of this study point to that direction. The distribution of microbiologically positive animals among the herds was not statistically significant but this can be attributed to the relatively small size of the groups. The overall prevalence was 49.38%. Most researchers report prevalence values up to 50%. Herds A (50%), C (33.33%), E (31.25%) and F (50%) are in agreement to that, but herds B (70%), D(70%) and G (60%) are not, a fact that shows that small variations in herd management are a key factor. Overall differences as seen in Table 2 among breeds were not statistically significant, a result that stresses the fact that bad management practices can overwhelm the idiosyncratic resistance of a breed (if any) to infectious factors.

Table 2. CMT scores versus microbiological status.

Herd	CMT (-) or (\pm)		CMT positive score		Total
	Bact (-)	Bact (+)	Bact (-)	Bact (+)	
A	20	4	0	16	40
B	12	28	0	0	40
C	20	0	20	20	60
D	0	0	12	28	40
E	8	4	36	16	64
F	12	4	8	16	40
G	0	4	16	20	40
Total	72	44	92	116	324

Staphylococcus spp. was the most prevalent genus, represented by 9 species (Table 3). It was isolated from 108 animals in total (33.33%), distributed among all herds. Similar findings have been reported by other researchers [20,29] and probably this genus originates from the hands of the milkers and the skin of the udder [30]. Skopelos breed seems to be significantly ($\chi^2=6.97$, 1df, $p < 0.05$) more vulnerable (48 out of 80 animals, 60.00%) than the indigenous breed (60 out of 244 animals, 24.59%). In total 48 out of 108 animals (44.44%) with subclinical infection by *Staphylococcus* spp. belonged to the Skopelos breed (OR=4.6, CI 05%: 1.58-13.35). *S. aureus* was the most prevalent genus, isolated from 40 out of the 108 animals (37.03%). This spe-

cies is considered the most important pathogen in mastitis in dairy goats [29,31]. Skopelos breed was significantly ($\chi^2=6.00$, 1df, $p<0.05$) most vulnerable with 8 animals out of 12 (75%), while the local breed seems more resistant (2 out of 15 animals, 13.33%) (OR=13, CI 95%: 1.919-88.06). Resistance and vulnerability concerning other Staphylococcus species (Coagulase Negative Staphylococci) seem to depend on the herd. Herd C was the most resistant with only 4 animal infected (by *S. warneri*). *S. chromogenes* was isolated only in herd D, while *S. xylosus* and *S. lentus* were isolated only from herd E. It is noteworthy to mention that only 4 animal in herd G had a mixed staphylococcal infection caused by *S. caprae* and *S. simulans* (in different half-udder). The location of the infection is also an important factor because 28 animals were infected in both half-udders (25.92% of the infected), while 56 animals (51.85%) were infected in the right half-udder and the rest 24 animals (22.22%) were infected in the left half-udder. *S. epidermidis* although is considered the most frequently isolated species among the CNS [4,16,17,32] was not isolated in the present study.

Table 3. Prevalence of Staphylococcus infection of different udder halves.

Herd	Species	Animals	Half-udder
A	<i>S. aureus</i>	16	†L+R)*, 12R)4
	<i>S. auricularis</i>	8	L+R), 4R)4
B	<i>S. aureus</i>	8	L+R), 4R)4
	<i>S. haemolyticus</i>	8	‡L+R), 4L)4
	<i>S. caprae</i>	4	4L
C	<i>S. warneri</i>	4	4L
D	<i>S. caprae</i>	4	(L+R)4
	<i>S. chromogenes</i>	16	L+R), 12R)4
E	<i>S. lentus</i>	4	4L
	<i>S. xylosus</i>	4	4R
F	<i>S. aureus</i>	16	8R,8L
	<i>S. haemolyticus</i>	4	4R
	<i>S. simulans</i>	4	4R
G	<i>S. caprae</i>	4	4R
	<i>S. caprae/S. simulans</i>	4	(R+L)4
Total	species 9	108	L+R), 56R, 24L)28

*: (L+R); Left and Right half udder, †: R; Right half udder, ‡: R; Left half udder

Table 4 presents the prevalence of subclinical infections caused by *Streptococcus* spp strains. The prevalence of this genus was much lower than the one of Staphylococci. Only 16 animals were infected (4.93% of the total), by 16 different species, distributed in 4 herds. In herds B and C the infections were mixed either with *S. aureus* or with another microorganism. None of the species mentioned in the relevant literature e.g. *Str. uberis*, *Str. agalactiae*, *Str. disgalactiae* and others [29] was isolated in the present study. A possible explanation is that these species are pathogenic and cause clinical mastitis, while the objective of this study was

not focused on animals with clinical signs of mastitis but on healthy ones with subclinical mastitis. Skopelos breed seems resistant to this genus since it was not isolated from any animal belonging to herds A and F. As in the case of Staphylococci, Streptococci were also isolated mainly from the right half-udder.

Table 4. Prevalence of *Streptococcus* spp.

<i>Streptococcus</i> spp.	Herd	Animals	Half-udder	Multiple infections
<i>Streptococcus suis</i>	B	4	R	<i>S. aureus</i>
<i>Streptococcus parasanguis</i>	C	4	R	<i>Kocuria kristinae</i>
<i>Streptococcus pneumoniae</i>	D	4	L+R	-
<i>Streptococcus thoralensis</i>	E	4	L	-

Table 5 presents the prevalence of other microorganisms, such as saprophytic microorganisms as well isolated from milk samples. In total 15 species were isolated from 64 out of 324 animals (19.75%), distributed in all herds. Skopelos breed was found much less sensitive (8 animals out of 80, 10%) than the local breed (56 animals out of 244, 22.95%). In 32 of the 64 animals which were subclinically infected by others microorganisms except the common pathogens and saprophytes, the infection was multiple with Staphylococci or Streptococci, while in the other 32 the infection was exclusively caused by others microorganisms (not the most common pathogens bacteria). This prevalence (9.87%) is almost double than the one of Streptococci (4.93%), who are considered as the second most frequently present bacteria in goat milk after Staphylococci [29]. This finding suggests that the saprophytes and the rarely isolated pathogens such as *Bacilli*, are capable of causing intramammary infection in higher rate, than usually expected. Small wounds of the teats, minor injuries of the udder, bad milking practices and low hygienic status of the facilities may be the source of such infections [33]. Saprophytic microorganisms are generally rather overlooked. They originate from the environment and the skin of the udder and are considered as opportunistic pathogens of minor importance [34,35]. However, the findings of the present study suggest otherwise.

Table 5. Prevalence of other pathogens, opportunistic pathogens and saprophytes.

Herd	Species	Animals	Half-udder	Multiple infection
A	<i>Alloicoccus otitis</i>	4	4L	Yes
B	<i>Bacillus</i> spp.	8	4L,4R	Yes
	<i>Bacillus</i> spp., <i>Acinetobacter iwoffii</i> , <i>Moraxella</i> spp.	4	4R	Yes

	<i>Sphingomonas paucimobilis</i>	4	4R	Yes
C	(<i>Kocuria varians</i> , <i>Sphingomonas paucimobilis</i> , <i>Bacillus spp</i>)/ <i>Sphingomonas paucimobilis</i>	4	4(L+R)	No
	<i>Klebsiella pneumonia</i> , <i>Rothia mucilaginosa</i>	4	4R	No
	<i>Kocuria kristinae</i> , <i>Sphingomonas paucimobilis</i>	4	4R	Yes
D	<i>Pantoea spp.</i>	4	4R	Yes
E	<i>Bacillus spp.</i>	4	4(L+R)	No
	<i>Bacillus spp.</i>	4	4R	No
F	<i>Acinetobacter iwoffii</i>	4	4L	No
G	<i>Bacillus spp.</i>	4	4R	Yes
	<i>Bacillus spp.</i> , <i>Dermacoccus spp.</i> , <i>Kytococcus spp.</i> , <i>Kocuria cosea</i>	4	4R	No
	<i>Micrococcus luteus</i> , <i>Micrococcus lylae</i> , <i>Sphingomonas paucimobilis</i>	4	4L	No
	<i>Bacillus spp.</i>	4	4R	No
Total	15 species	64	8(L+R),40R, 8L	Yes: 32 / No: 32

As in the case of Staphylococci and Streptococci, most opportunistic pathogens were isolated from the right half-udder. There is not any anatomical reason for this observation beyond the fact that half-udders are and function as semi-autonomous units. Various researchers described this phenomenon but were not able to offer an explanation. In cases where a milking machine was used the cause can be attributed to the initial settings of the machine. However, in cases of manual milking the explanation might be different. Careful inspection of Tables 3, 4 and 5 reveals that the microbiological positivity of the right or the left half-udder varies from herd to herd according to the cause of the infection. For example, in Herd C one left half-udder is infected by *S. warnerii*, while in the same herd 3 left half-udders are infected by saprophytes. It seems that there is not any particular pattern.

The finding of this study that more right half-udders than left-half-udders were infected suggests that other factors are also implicated. Animals that are milked first may have lower possibility of getting infected than the ones who are milked last when the farmer is tired and tends to be less

careful with the handling or the cleaning of the teats. Also right handed farmers (or left handed ones) tend to squeeze harder the teat that they grab with their right (or left) hand, respectively.

Most of the milk produced by goats is used for cheese production. Mastitic milk has elevated levels of plasmin, a proteolytic enzyme which converts casein into whey protein. Since casein is required for cheese production, mastitic milk yields less cheese than does non-mastitic milk (on a percentage basis). [32,36] compared curd yield and clotting time, as criteria of the quality of milk for cheese production, in the milk of both sheep and goats, experimentally infected in one udder-half with Coagulase Negative Staphylococci, with the milk produced from their uninfected half. Uninfected sheep and goat milk had curd yields of 30% and 23%, respectively, while milk from the infected halves of sheep and goats had curd yields of 14% and 20%, respectively. CNS infection also increased clotting time from 413 seconds in uninfected milk to 909 seconds in infected sheep milk and from 167 seconds in uninfected milk to 295 seconds in infected goat milk. It was concluded that beyond its effect on milk yield, CNS mastitis also has an effect on cheese yield of sheep and goat milk intended for this purpose [32,36]

Subclinical mastitis is a serious problem for most goat herds in Greece and it can affect their overall milk production. Despite the results of the ongoing research on the microorganisms causing such infections, differences in management practices may also affect directly or indirectly the health of the animal as well the health of the udder.

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