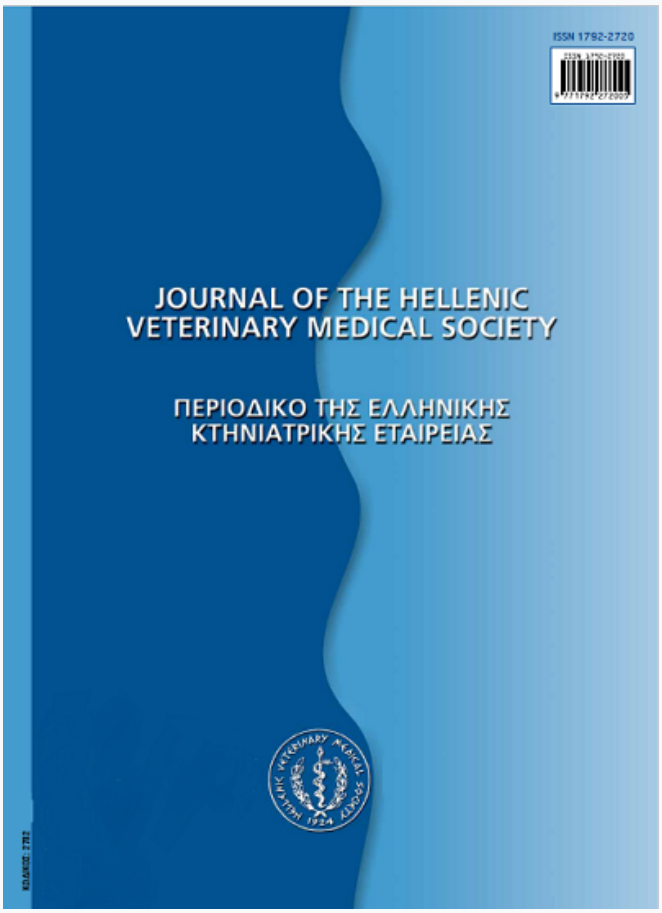
Journal of the Hellenic Veterinary Medical Society

Vol 67, No 2 (2016)



The bacterial flora of the udder of goats

A. TZORA, J. SKOUFOS, A. TSINAS, K. FOTOU, A. KARAMOUTSIOS, Z. KALYVA, K. NIKOLAOU, G. C. FTHENAKIS

doi: 10.12681/jhvms.15628

To cite this article:

TZORA, A., SKOUFOS, J., TSINAS, A., FOTOU, K., KARAMOUTSIOS, A., KALYVA, Z., NIKOLAOU, K., & FTHENAKIS, G. C. (2018). The bacterial flora of the udder of goats. *Journal of the Hellenic Veterinary Medical Society*, *67*(2), 99–108. https://doi.org/10.12681/jhvms.15628

J HELLENIC VET MED SOC 2016, 67(2): 99-108
ΠΕΚΕ 2016, 67(2): 99-108

Review article
Ανασκόπηση

The bacterial flora of the udder of goats

Tzora A.[1], Skoufos J.[1], Tsinas A.[1], Fotou K.[1], Karamoutsios A.[1], Kalyva Z.[1], Nikolaou K.[1], Fthenakis G.C.[2]

[1]TEI of Epirus. Department of Agriculture Technology, Division of Animal Production, 47100 Arta, Greece

[2]Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece

Η βακτηριακή χλωρίδα του μαστού των αιγών

Τζώρα Α.[1], Σκούφος Ι.[1], Τσίνας Α.[1], Φώτου Κ.[1], Καραμούτσιος Α.[1] Καλύβα Ζ.[1], Νικολάου Κ.1, Φθενάκης Γ.Χ.[2]

[1]ΤΕΙ Ηπείρου, Τμήμα Τεχνολογίας Γεωπονίας, 47100 Άρτα

[2]Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας, 43100 Καρδίτσα

ABSTRACT. Aims of the study were to identify microbial flora in healthy caprine udders and to evaluate possible sources of principal caprine mastitis pathogens. Samples of teat duct material and mammary secretion from goats in two farms (flock A, polyparous n=30; flock B, polyparous n=60) were collected from both glands, four times during the lactation period. Subsequently, bacteriological investigation took place from 200 udders of slaughtered goats (n=100). Mammary gland skin swabs were examined bacteriologically from one farm (flock C, polyparous n=60) four times, one before and three during the lactation period. In all milk samples, cytological examinations were negative. Conventional bacteriological techniques were used. Bacterial contamination was found in 93% of samples from the skin of the teat, in 44% of teat duct scraping samples, in 6% of teat duct material samples and in 4% of mammary secretion samples. Different bacterial species were isolated, mainly coagulase negative staphylococci. This investigation showed smaller proportion of contamination in the teat canal than in the outer surface of the teat skin, indicating that teat provides innate defensive systems against bacterial invasions.

Keywords: bacterial flora, goats, mammary defence, mastitis, milk, sheep, teat

Correspondence: A. Tzora,
Department of Agriculture Technology,
TEI of Epirus, 47100 Arta, Greece.
E-mail: tzora@teiep.gr

Αλληλογραφία: Α. Τζώρα,
Τμήμα Τεχνολογίας Γεωπονίας,
ΤΕΙ Ηπείρου, 47100 Άρτα.
E-mail: tzora@teiep.gr

Date of initial submission: 29 August 2014
Date of revised submission: 30 September 2014
Date of acceptance: 2 October 2014

Ημερομηνία αρχικής υποβολής: 29 Αυγούστου 2014
Ημερομηνία αναθεωρημένης υποβολής: 30 Σεπτεμβρίου 2014
Ημερομηνία αποδοχής: 2 Οκτωβρίου 2014

ΠΕΡΙΛΗΨΗ. Σκοπός της μελέτης ήταν η ταυτοποίηση της μικροβιακής χλωρίδας του μαστικού αδένα σε υγιείς αίγες και ο καθορισμός των κύριων αιτιολογικών παραγόντων μαστίτιδας σε αυτά τα ζώα. Έγινε συλλογή δειγμάτων γάλακτος και υλικού θηλαίου πόρου μαστικού αδένα σε πολυτόκες αίγες σε δύο εκτροφές, συνολικά σε 90 ζώα. Συλλέχθηκαν δείγματα και από τους δύο μαστικούς αδένες, τέσσερις φορές κατά τη διάρκεια της γαλακτικής περιόδου. Επιπλέον, έγινε μικροβιολογική εξέταση σε 200 μαστούς αιγών από σφαγείο. Τέλος, συλλέχθηκαν δείγματα, με βαμβακοφόρο στυλεό, από το δέρμα του μαστού για βακτηριολογική εξέταση από 60 πολυτόκα ζώα, μια φορά κατά την ξηρή περίοδο και τρείς φορές κατά τη γαλακτική περίοδο. Σε όλα τα δείγματα η κυτταρολογική εξέταση ήταν αρνητική. Απομονώθηκαν βακτήρια από το 93% των δειγμάτων της εξωτερικής επιφάνειας της θηλής, το 44% των δειγμάτων ξεσμάτων θηλαίου πόρου, το 6% των δειγμάτων υλικού θηλαίου πόρου και το 4% των δειγμάτων γάλακτος. Ταυτοποιήθηκαν διάφορα γένη βακτηρίων, κυρίως σταφυλόκοκκοι, ειδικότερα δε πηκτάση-αρνητικοί σταφυλόκοκκοι. Η μελέτη απέδειξε μικρότερη αναλογία μόλυνσης στο θηλαίο πόρο του μαστού συγκριτικά με την εξωτερική επιφάνεια της θηλής, αλλά μεγαλύτερη από αυτήν στο γάλα, υποδηλώνοντας ότι η θηλή έχει εγγενείς αμυντικούς μηχανισμούς πού εμποδίζουν τη διείσδυση των βακτηρίων προς το μαστικό παρέγχυμα μέσω του θηλαίου πόρου.

Λέξεις ευρετηρίασης: αίγες, αμυντικοί μηχανισμοί, βακτηριακή χλωρίδα, γάλα, θηλή, μαστίτιδα, πρόβατο

INTRODUCTION

In studies of bacterial mastitis in does, it is useful to obtain information regarding the bacterial flora of the udder. Such information is helpful in two ways: first, it is of interest to learn if bacteria constituting normal flora of various sites at the udder are potential pathogens for the caprine mammary gland and second, it is interesting, from the epidemiological viewpoint, to know whether some of the organisms commonly associated with caprine mastitis, reside in the udder of healthy does.

There is no information available on the bacterial flora in healthy caprine udders, although, in other animal species, various studies have highlighted that. In cows, it has been reported, long time ago, that the principal bacteria constituting the bacterial flora of the skin of the teat are various coagulase-negative staphylococci, as well as *Staphylococcus aureus* and various *Streptococcus* spp. (Edwards and Jones, 1966; Cullen and Hebert, 1967). In ewes, Mavrogianni et al. (2007) studied extensively the bacterial flora of the teat of ewes and reported that *Mannheimia haemolytica* and *Staphylococcus* spp. were the principal organisms. In sows, Kemper and Preissler (2011) found that coagulase-negative staphylococci and *Streptococcus* spp were the most frequently isolated bacteria from the teat duct of post-parturient sows, whilst *Escherichia coli* and *Klebsiella aerogenes*, the principal porcine mammary pathogens (Jones, 1971). Finally, in women, various studies have shown that milk of healthy individuals was seldom sterile, with the predominant and most frequently isolated bacteria reported to be coagulase-negative staphylococci (Gavin and Ostovar, 1977; West et al., 1979).

Since information is not available on the bacterial flora of the udder of does and since this information is germane in the understanding of caprine mastitis, the three investigations described herebelow have been undertaken. Their objectives were as follows: (i) to study distribution and identity of the bacterial flora of the caprine udder and (ii) to study possible residence of principal mastitis pathogens as a flora of the udder.

MATERIALS AND METHODS

Study design

Investigation I. Bacteriological investigation of mammary secretion of lactating does

Two dairy herds (A and B) were studied; herd A included 250 does of Skopelos breed and herd B included 430 *Capra prisca* does. Animals were maintained according to the semi-intensive system, with a lactation period of nine months. They were grazing and also fed commercially prepared concentrates, supplemented with hay. In each herd, 30 (A) and 60 (B) polyparous animals, selected at random, were included into the investigation.

Samples were obtained from both glands of each doe four times during a lactation period. The first sample was obtained 4 to 5 days after kidding and the second one month later, i.e., when animals were still suckling their kids. The third sample was obtained on the fifth month and the final sample was collected on the eighth to ninth month of lactation period, i.e., when animals were hand-milked.

For sample collection, each doe was cast and restrained and her mammary glands were examined. The glands were observed, palpated and compared with each other. Their shape, size, consistency, temperature and any abnormalities were assessed and recorded. Any doe with abnormalities was excluded from the investigation. The teat orifice and skin were disinfected with 'clinical iodine'. A sterile plastic fine 2 mm-long 21G catheter (Abbocath) (Mavrogianni et al., 2006a) was inserted into the teat and moved from the left to right, in order to sample the mucosa. Then, the first squirt of secretion (foremilk) was collected into a sterile glass bijou-bottle. Finally, 12 to 15 ml of milk were collected carefully into a sterile Universal bottle. After collection was completed, the teat orifice and skin were disinfected again with clinical iodine.

After collection, all samples were maintained in 4 ^{o}C until processing. All procedures were completed within a maximum of three hours after collection of the samples.

Investigation II. Bacteriological investigation of the teats of udder of slaughtered does

The udders of 100 dairy does were collected from a local abattoir. No specific information was available about origin and disease history of these animals.

Transport of the material to the laboratory took place within one hour of slaughter of the animals. Dissection of the teats started immediately after the arrival of the material at the laboratory, but took up to 2 hours to complete. The mammary glands were maintained at 4 ^{o}C, until processing.

Before the mammary glands and teats were dissected, they were carefully examined, by observation and palpation for the presence of any abnormalities. Glands with abnormalities were discarded. A red-hot spatula was applied on the dorsal surface of each gland and a piece of mammary tissue was removed aseptically. This tissue was immediately rubbed gently on 5% sheep blood Columbia agar. Then, each teat was ligated at its base with strong nylon and a simple mammillectomy was performed. After both teats were removed, the mammary glands were incised many times to facilitate search for gross lesions. If any were found, the teats were not dissected.

Each teat was pinned on a wax board and, after sterilizing its surface with a red-hot spatula, the skin was incised lengthwise. Then, the subcutaneous tissues were incised, so as to expose the mucosa of the teat cistern (*sinus papillaris*). Subsequently, the teat duct (*ductus papillaris*) was incised and exposed. These were maintained in the stretched position by repining the skin of the teat on the board. A new sterile scalpel blade was moistened with a drop of Todd-Hewitt broth and was used for scraping the mucosa of the teat cistern. Another sterile scalpel blade was moistened and was used to scrape the internal surface of the wall of the teat duct.

Investigation III. Bacteriological investigation of the skin of the udder of lactating does

One dairy herd (C), with 380 dairy does of Skopelos breed was studied. Animals were maintained according to the semi-intensive system, with a lactation period of nine months. They were grazing and also fed commercially prepared concentrates, supplemented with hay. In total, 60 polyparous animals, selected at random, were included into the investigation.

Samples were obtained once two months before kidding and thrice during a lactation period. The first sample was obtained 4 to 5 days after kidding and the second one month later, i.e., when animals were still suckling their kids. The third sample was obtained on the fifth month of lactation period, i.e., when animals were hand-milked. Initially, using two different cotton swabs, moistened in Todd-Hewitt broth, the skin of each mammary gland (one swab for each gland) was swabbed. Then, using another two cotton swabs, the lateral surface of each teat (one swab for each teat) was swabbed. Finally, each teat was tightly restrained between two fingers and the lower surface

of the teat was sampled by using, on each occasion, a different swab. After swabbing was finished, the mammary glands were examined, as described above. Any does with abnormalities were excluded from the investigation. Finally, teat duct material and milk were collected, as described above, on sampling occasions during the lactation period.

After collection, all samples were maintained in 4 °C until processing. All procedures were completed within a maximum of three hours after collection of the samples.

Bacteriological examination

The catheters, the scalpel blades and the swabs were rubbed on 5% sheep blood Columbia agar and moved on there. A loopful of each foremilk or milk sample (0.01 ml) was taken and inoculated onto 5% sheep blood Columbia agar. The inoculated media were incubated in an aerobic environment at 37 °C and examined for up to 72 hours. If bacteria grew, the characteristics of the colonies were described and their growth recorded. They were identified according to the methods of Cowan (1974).

Cytological examination

In all milk samples, the California Mastitis Test was applied after completion of the bacteriological examination (Fthenakis, 1995). Finally, milk films were prepared and stained by the Giemsa technique for microscopic examination.

RESULTS

Investigation I

All animals into the study were clinically healthy. No CMT scores ≥1 were detected; in milk films, only scarce macrophages were observed. Detailed results of bacteriological examinations are in Tables 1 and 2.

Investigation II

All teats originated from mammary glands with no gross abnormalities. Bacteria were not isolated from any mammary tissue sample. In total, 200 teats were sampled at each of two sites (teat cistern and teat duct), i.e. 400 sites. From 42 (42%) does, no bacteria were isolated, from 23 (23%) does bacteria were isolated from only one teat and from 35 (35%) does bacteria were isolated from both teats. Detailed results of the isolation of bacteria from the udder of does examined are in Table 3.

Bacteria were isolated from 93 (47%) of the 200 teats. In 6 (3%) teats bacteria were isolated only from the teat cistern, while in 44 teats (22%) bacteria were isolated only from the teat duct. In 43 (22%) teats bacteria were isolated from both the teat cistern and the teat duct; in 38 (88%), of these

Table 1. Results of bacteriologically positive findings from samples collected from the udder of does in two dairy herds.

	No of bacteriologically positive samples		
Sampling occasion	Teat duct material samples	Foremilk samples	Milk samples
Herd A			
1st (n=60)	5	4	0
2nd (n=60)	5	4	0
3rd (n=60)	3	2	0
4th (n=60)	2	2	0
Total (n=240)	15 (6%)	12 (5%)	0
Herd B			
1st (n=120)	7	4	0
2nd (n=120)	8	4	0
3rd (n=120)	4	4	1
4th (n=120)	5	6	1
Total (n=480)	24 (5%)	18 (4%)	2 (<1%)

43 teats, the same bacteria were isolated from the teat cistern and the teat duct, but in some instances fewer bacteria were isolated from the cistern. The results of the isolation of bacteria from the teats sampled are in Table 4.

Bacteria were isolated from 49 (25%) samples from teat cisterns; coagulase-negative staphylococci were isolated from 16% of the samples and accounted for 65% of the bacterial isolates. Bacteria were isolated from 87 (44%) samples from teat ducts; coagulase-negative staphylococci were isolated from 29% of the samples and accounted for 65% of the bacterial isolates. Results of the identity of bacteria isolated from scrapings are in Table 5.

In total, there were 136 bacterial isolates from all samples and of these, 89 (65%) were coagulase-negative staphylococci. These organisms were isolated from 44 (44%) does. In 22 does, they were isolated from only one teat and in 22 does, they were isolated from both teats.

Investigation III

All 60 does included in this investigation were clinically healthy throughout the study. No CMT scores ≥1 were detected in milk samples; in milk films, only scarce macrophages were observed.

In total, 480 swab samples from the skin of the mammary glands were examined. Bacteria, always

Table 2. Frequency of isolation of bacterial species isolated from samples collected from the udder of does in two dairy herds.

Bacterial species	No of bacteriologically positive samples		
	Teat duct material samples	Foremilk samples	Milk samples
Herd A			
Staphylococcus simulans	6	5	0
Staphylococcus epidermidis	5	4	0
Staphylococcus caprae	3	3	0
Mannheimia haemolytica	1	0	0
Total	15	12	0
Herd B			
S. simulans	5	4	1
S. epidermidis	7	7	0
S. caprae	5	5	0
S. xylosus	3	2	0
M. haemolytica	2	0	0
Escherichia coli	2	0	0
Micrococcus sp.	0	0	1
Total	24	18	2

Table 3. The results of the isolation of bacteria from the udder of does examined in investigation II.

Does from which animals udders were sampled	100
Does with all 4 sampling sites in their udder bacteriologically negative	42
Does with at least one sampling site in their udder bacteriologically positive	58
Does with only one sampling site in their udder bacteriologically positive	13
Does with only one teat duct bacteriologically positive	12
Does with only one teat cistern bacteriologically positive	1
Does with only two sampling sites in their udder bacteriologically positive	24
Does with both bacteriologically positive sampling sites in the same teat	10
Does with both bacteriologically positive sampling sites in different teats	14
Does with three sampling sites bacteriologically positive	9
Does with all four sampling sites bacteriologically positive	12

Table 4. The results of the isolation of bacteria from teats sampled on investigation II.

Teats with teat duct negative and teat cistern negative	107
Teats with teat duct negative and teat cistern positive	6
Teats with teat duct positive and teat cistern negative	44
Teats with teat duct positive and teat cistern positive	43
Teat cistern with the same bacteria as teat duct and in similar quantity	16
Teat cistern with the same bacteria as teat duct but in smaller quantity	22
Teat cistern with the same bacteria as teat duct and with other bacteria	2
Teat cistern with bacteria differing from those in the teat duct	3

Table 5. Frequency of isolation of bacterial species isolated from scrapings from udders of does.

Bacteria isolated	Number of samples, from which isolated
Scrapings from teat cisterns	
S. epidermidis	8
S. simulans	7
S. caprae	6
S. xylosus	5
S. aureus	4
S. chromogenes	4
Bacillus sp.	3
S. sciuri	2
B. licheniformis	2
Streptococcus sp.	2
Trueperella pyogenes	2
Erwinia sp.	1
E. coli	1
M. haemolytica	1
Morganella morgani	1
Total	49
Scrapings from teat ducts	
S. epidermidis	14
S. simulans	12
S. caprae	11
S. xylosus	9
Bacillus sp.	8
S. chromogenes	6
S. aureus	5
S. sciuri	5
Streptococcus sp.	4
B. licheniformis	3
T. pyogenes	3
B. cereus	2
E. coli	2
Erwinia sp.	1
M. haemolytica	1
M. morgani	1
Total	87

Table 6. Results of bacteriologically positive findings from samples collected from the udder of does.

	No of bacteriologically positive samples	
Sampling occasion	Teat duct material samples	Milk samples
Herd C		
1st (n=120)	4	0
2nd (n=120)	6	0
3rd (n=120)	4	0
4th (n=120)	2	0
Total (n=480)	16 (3%)	0 (0%)

in mixed culture, were isolated from all these samples. Of the 480 samples, only three bacterial species were isolated from 53 (11%) of them, while in the remainder at least four to seven bacterial species were isolated.

In total, 480 swab samples from the skin of the lateral surface of the teats were examined. Bacteria, always in mixed culture, were isolated from all these samples. Of the 480 samples, only three bacterial species were isolated from 82 (17%) of them, while in the remainder at least four to six bacterial species were isolated.

Finally, 480 swab samples from the skin of the surface of the teat, where the orifice is found, were examined. Bacteria, mostly in mixed culture, were isolated from 448 (93%) of these samples. In glands, from whose secretion bacteria were isolated in pure culture, similar bacteria were isolated from the lower surface of the teat.

Coagulase-negative staphylococci and *Bacillus* spp. were the most frequently isolated bacteria. However, other bacterial species: *Acinetobacter anitratus*, *Corynebacterium* spp., *E. coli*, *Micrococcus* spp., *Streptococcus* spp., *T. pyogenes* were also frequently isolated. *S. aureus* was isolated from 94 (7%) samples; of these, 45 were udder skin swabs, 43 were teat lateral surface swabs and 6 were teat lower surface swabs; moreover, of these 94 samples, 9 were collected during pregnancy and 85 during the lactation period of does (45 in both occasions during the suckling period and 40 in the one occasion during the milking period). *M. haemolytica* was isolated from 5 (<1%) samples collected during the suckling period; of these, 3 were teat lateral surface swabs and 2 were teat lower surface swabs.

Detailed results of bacteriological examinations are in Tables 6 and 7.

DISCUSSION

In investigations I and III, the mammary glands of all does sampled were healthy, as confirmed by results of clinical evaluation, as well as milk bacteriological and cytological examinations. Moreover, in investigation II, where udders from an abattoir were taken and sampled, no gross abnormalities were identified in any of these udders. Therefore, the results indicate the distribution and identity of the bacterial flora of healthy caprine udders and the possible sources of principal mastitis pathogens in such cases.

The bacterial flora of the skin of the mammary gland and of the skin of the teat of ewes

We used the swabbing technique, because of its simplicity as related to the information required. A plethora of bacteria was isolated from the skin of the mammary glands and the skin of the teats of does in herd C. Organisms isolated are similar to those identified in the skin of the udder of cows or ewes (Cullen and Herbert, 1967; Mavrogianni et al., 2007). *S. aureus* was isolated more frequently during the milking period and *M. haemolytica* was isolated only during the suckling period. Of the various organisms isolated, *S. aureus* and coagulase-negative staphylococci constitute important causal agents of caprine mastitis (Contreras et al., 2007; Jakeen et al., 2013; Tzora et al., 2014), whilst *M. haemolytica* is of lesser significance (Anderson et al., 2002). The results provide clear evidence that these organisms are part of the bacterial flora of the skin of the udder, from where they may invade the teat and subsequently the mammary parenchyma.

Staphylococci can colonise the skin of the udder being transmitted from the hands of milkers (Zadoks et al., 2002; Rosengren et al., 2010). Subsequently,

Table 7. Frequency of isolation of bacterial species isolated from samples collected from the udder of does.

	No of bacteriologically positive samples	
Bacterial species	Teat duct material samples	Milk samples
Herd C		
S. epidermidis	6	0
S. simulans	5	0
S. caprae	2	0
S. aureus	1	0
M. haemolytica	1	0
Micrococcus sp.	1	0
Total	16	0

these organisms may transmit from one doe to another by means of clusters of the milking machine or the hands of milkers (Lee et al., 2012; Quigley et al., 2013). *M. haemolytica* has been shown to be transferred from the tonsils of the sucking lamb to the teats of their dam (Fragkou et al., 2011). In our study, isolation of the organism from the skin of the teat during the early stage of a lactation period indicates that the same mode of transmission likely occurs in goat herds. In general, respiratory or mammary infections by *Mannheimia* in goats have not been studied extensively.

The bacterial flora of the teat duct and teat cistern of does

Bacterial invasion through the teat constitutes the first phase in the pathogenesis of mastitis (Mavrogianni et al., 2005). Introduction of bacteria, however, at the teat is by no means sufficient to result in mastitis. In ewes, Mavrogianni et al. (2005; 2006b) have shown that the teat acts as a barrier to mammary infection.

In investigations I and III, samples of teat duct material were collected by means of Abbocath fine catheters. In investigation II, scrapings from teat ducts ant teat cisterns of slaughtered does were examined bacteriologically. The results suggested the bacteria present in the walls of the internal anatomical structures of the teat. Moreover, in investigation III, swabs of the surface of the teat were obtained. In general, this approach provided hints regarding risk of mammary infection by organisms in close vicinity to the mammary gland.

Bacteria were isolated from 93% of samples from the skin of the lower surface of the teat, from 44% of teat duct scraping samples, from 6% of teat duct material samples and from 4% of foremilk samples. The results confirm bacteria frequently reside inside the teats of does. Although all animals have been found to be colonised with bacteria in the outer surface of the teat, bacteria were isolated from significantly less samples from the inside of the teat. This indicates that various antimicrobial functions inhibit colonisation of the teat. The larger proportion of bacterial isolation from slaughtered animals in comparison to live does (i.e., teat duct scrapings from slaughtered does *versus* teat duct material collected by means of fine catheters) indicates that after slaughter (i.e., death), when these functions are terminated, rapid bacterial multiplication occurs. Finally, the smaller proportion of bacteriologically positive foremilk samples recorded in investigation I indicates that bacteria remain attached on the teat wall and only occasionally pass and can be found in milk.

Although bacteriologically positive teat duct material and foremilk samples were obtained, milk samples from the same animals were bacteriologically negative. This supports the theory regarding defence functions in the teat, as these mechanisms protect ascent of the bacteria present in the teat into the mammary parenchyma. Alternatively, one may propose that the particular strains isolated from the teat duct material samples lacked virulence factors which would allow them to ascend into the mammary parenchyma.

The bacteria isolated from each of the sites sampled in each doe were not always similar for the left and the right gland. Therefore, although mammary glands are exposed to the same environment, there may be variations in the type of bacteria present in

the two mammary glands of the same animals.

The presence of bacteria inside the teat constitutes a constant reservoir of infection for the mammary tissue and mastitis might develop, if the equilibrium between bacteria and host defences shifts to the bacterial side, due to a possible reduction in the efficiency of the defence systems of the host.

CONCLUDING REMARKS

The results of the present study confirm that a plethora of bacteria, mainly coagulase-negative staphylococci, are present in the udder skin and inside the teat of does. These organisms can be sources of infection for the mammary gland of these animals. The teat provides defensive systems against bacterial invasions. There is a scope to study the patterns of such infections and to elucidate these mechanisms, as it will help to formulate control strategies.

ACKNOWLEDMENTS

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

Anderson DE, Hull BL, Pugh DG (2002) Diseases of the mammary gland. In: (ed.: Pugh DG) Sheep and Goat Medicine. Saunders, Philadelphia, pp. 341-358.

Contreras A, Sierra D, Sánchez A, Corrales JC, Marco JC, Paape MJ, Gonzalo C (2007) Mastitis in small ruminants. Small Rumin. Res. 68, 145-153.

Cowan ST (1974) Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd edn. Cambridge University Press, Cambridge.

Cullen GA, Hebert CN (1967) Some ecological observations on micro-organisms inhabiting bovine skin, teat canals and milk. Br. Vet. J. 123, 14–25.

Edwards SJ, Jones GW (1966) The distribution and characters of coagulase-negative staphylococci of the bovine udder. J. Dairy Res. 33, 261-270.

Fragkou IA, Gougoulis DA, Billinis C, Mavrogianni VS, Bushnell MJ, Cripps PJ, Tzora A, Fthenakis GC (2011) Transmission of *Mannheimia haemolytica* from the tonsils of lambs to the teat of ewes during sucking. Vet. Microbiol. 148, 66-74.

Fthenakis GC (1995) California Mastitis Test and Whiteside Test in diagnosis of subclinical mastitis of dairy ewes. Small Rumin. Res. 16, 271-276.

Gavin A, Ostovar K (1977) Microbiological characterization of human milk. J. Food Prot. 40, 614-616.

Jakeen El-Jakee K, Noha Aref E, Gomaa A, Mahmoud El-Hariri D, Hussein Galal M, Sherif Omar A, Samir A (2013) Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals. Int. J. Vet. Sci. Med. 1, 74-78.

Jones JET (1971) Reflections on post-parturient diseases associated with lactational failure in sows. Vet. Rec. 89, 72-77.

Kemper N, Preissler R (2011) Bacterial flora on the mammary gland skin of sows and in their colostrum. J. Swine Health Prod. 19, 112–115.

Lee SHI, Camargo CH, Gonçalves JL, Cruz AG, Sartori BT, Machado MB, Oliveira CAF (2012) Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. J. Dairy Sci. 95,7377–7383.

Mavrogianni VS, Cripps PJ, Fthenakis GC (2006a) Description and validation of a novel technique to study the bacterial flora of the teat duct of ewes. Small Rumin. Res. 66, 258-264.

Mavrogianni VS, Cripps PJ, Papaioannou N, Taitzoglou I, Fthenakis GC (2006b) Teat disorders predispose ewes to clinical mastitis after challenge with *Mannheimia haemolytica*. Vet. Res. 37, 89-105.

Mavrogianni VS, Cripps PJ, Fthenakis GC (2007b) Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. Prev. Vet. Med. 79, 163-173.

Mavrogianni VS, Fthenakis GC, Brooks H, Papaioannou N, Cripps PJ, Taitzoglou I, Brellou G, Saratsis P (2005) The effects of inoculation of *Mannheimia haemolytica* into the teat of lactating ewes. Vet. Res. 36, 13–25.

Quigley L, O Sullivan O, Stanton C, Beresford TM, Paul Ross R, Fitzgerald GF, Cotter PD (2013) The complex microbiota of raw milk. FEMS Microbiol. Rev. 37, 664–698.

Rosengren A, Fabricius A, Guss B, Sylvén S, Lindqvist R (2010) Occurence of food-borne pathogens and characterizations of *S. aureus* in cheese produced in farm-dairies. Int. J. Food Microbiol. 144, 263–269.

Tzora A, Skoufos I, Arsenos G, Giannenas I, Voidarou C, Fotou K, Kalyva Z, Fthenakis CG (2014) Etiology of subclinical mastitis in primiparous goats reared in the region of Epirus, Greece. Proceedings of the 65th Annual Meeting of the European Association of Animal Production (Copenhagen, Denmark), p. 313.

West PA, Hewitt JH, Murphy OM (1979) The influence of methods of collection and storage on the bacteriology of human milk. J. Applied Bacteriol. 46, 269–277.

Zadoks RN, Van Leeuwenand WB, Van Belkum A (2002) Comparison of *Staphylococcus aureus* isolates from bovine and human skin, milking equipment, and bovine milk by phage typing, pulsed-field gel electrophoresis, and binary typing. J. Clin. Microbiol. 40, 3894-3898.