THE BACTERIAL FLORA IN THE TEAT DUCT OF EWES CAN PROTECT AGAINST AND CAN CAUSE MASTITIS

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ABSTRACT

Objective. We carried out two experiments to study effects of bacterial flora into ovine teat duct to pathogenesis of mastitis. 1st experiment. 32 ewes were allocated into group A (ewes with coagulase-negative staphylococci [+++ growth] in the teat duct), B (ewes with coagulase-negative staphylococci [+ growth] in the teat duct) or C (ewes with *Bacillus* spp. in the teat duct) and subdivided into A1, B1, C1 (n=4; challenged by deposition of 1.250 cfu of *Mannheimia haemolytica* into the teat duct) or A2, B2, C2 (n=4; used as uninoculated controls); group D (n=8) had ewes with no bacteria in the teat duct, challenged also with *M. haemolytica*. There were less bacteriological isolations of flora (p=0.018) and challenge (p<0.05) organisms from A1 than from A2 and D ewes; pathological findings in A1 (summed up lesion score: 27/64) ewes were less severe than in D (summed up score: 36/64) ewes (p=0.038). No such findings were evident with B or C ewes (p>0.4).2nd experiment. Ewes (groups E and F, n=6) with coagulase-negative staphylococci (+ growth) in the teat duct, were used; ewes (group G, n=6) with no bacteria in the teat duct, were included. Teat chapping was applied in E and G ewes. All E ewes developed acute clinical mastitis within 24 h after chapping, although no challenge had been carried out; there were more bacteriological isolations of flora from E than from F or G ewes (p<0.001); pathological findings in E (summed up lesion score: 28/32) were more severe than in F (summed up lesion score: 3/32) or G (summed up lesion score: 14/32) ewes. Conclusions. We conclude that staphylococcal flora present in high numbers within the teat duct of ewes, provides some protection against invading bacteria. In case of decreased defence mechanisms in the teat, that flora can invade the mammary parenchyma and cause clinical mastitis. Acknowledgments. The project is co-funded by the European Social Fund & National Resources – EPEAEK II-PYTHAGORAS.

INTRODUCTION

The significance of the ovine teat as a defence mechanism against intramammary infections has been established [20]. Clinically healthy teats provided a substantial protection against *Mannheimia haemolytica* intramammary infection. Deposition of either of two isolates of *M. haemolytica* into the teat duct did not result to clinical mastitis, although an inflammatory reaction had been elicited. During that study, we observed lymphoid nodules at the border between teat duct – teat cistern; we postulated that these structures might play a protective role, as in histological sections from teats inoculated with the bacteria; they were hyperplastic with germinal activity. Apart from that, the bacterial flora residing into the teat duct of healthy ewes may also contribute to the protective role of the teat. In field studies, isolation of bacteria from clinically

healthy sheep teats was associated with observations of lymphoid nodules with germinal activity [23].

Coagulase-negative staphylococci are the principal organisms present as bacterial flora into sheep teats [10]. As staphylococci are confirmed aetiological agents of ovine mastitis [8], one may suggest that perhaps and under certain circumstances, flora organisms can also cause mastitis.

However, the above hypotheses have not been tested experimentally. The objective of the work presented in this paper was to investigate the role of the bacterial flora in the teat duct of ewes. Initially, we explored possible interactions between teat duct bacterial flora and invading microorganisms; subsequently, we studied whether teat lesions may predispose ewes to clinical mastitis caused by teat duct bacterial flora.

MATERIALS AND METHODS

Experimental design

– Overview

Two experiments were performed during this study. They were carried out under a licence for experimental procedures obtained from the Greek Ministry of Agriculture. In the first experiment, clinically healthy sheep teats with bacterial flora (coagulase-negative staphylococci or *Bacillus* spp.) into the duct were inoculated with an isolate of *M. haemolytica* (strain VSM08L). This strain had been isolated in Greece and was found to cause mastitis in ewes when inoculated directly into the gland cistern, whilst deposition into the duct or the cistern of clinically healthy teats resulted to subclinical mastitis [20]. The identity of the organism was initially established by means of conventional bacteriological techniques [6]. In the second experiment, clinically healthy sheep teats with bacterial flora (coagulase-negative staphylococci) into the duct were subjected to skin chapping lesions; no challenge was performed.

– Deposition of M. haemolytica into bacteriologically positive sheep teat ducts: Experiment I

Twenty-four, 3- to 5-years-old, Karagouniko-breed lactating ewes were included in the experiment. For selection of the ewes three, 2-day interval, examinations and samplings were carried out. Initially, a thorough clinical examination was carried out; special attention was paid to their mammary glands and teats, which were examined as described before [9, 20]. A sterile plastic fine catheter 2 mm long was inserted into the teat and moved from left to right, in order to sample the mucosa [21]. Then, mammary secretion samples (10 to 15 mL) were obtained. Selection of animals was based on concurrent presence of the following criteria at all three samplings: (i) clinically healthy mammary glands and teats; (ii) no bacterial isolation from mammary secretion; (iii) secretion CMT negative with minimal number of leucocytes in Giemsa-stained secretion films; (iv) bacterial isolation from teat catheter of one teat (left or right) in pure culture; (v) no bacterial isolation from teat catheter of the other teat. Allocation of animals into groups was carried out as follows; group A (n=8): isolation of coagulase-negative *Staphylococcus* sp. in heavy growth (+++), group B (n=8): isolation of coagulase-negative *Staphylococcus* sp. in mild growth (+), group C (n=8): isolation of *Bacillus* spp. in heavy growth (+++). Of the eight ewes allocated into each group, 4 (subgroups A1, B1, C1) were challenged and 4 (subgroups A2, B2, C2) were used as uninfected positive controls. Additionally, a group D (n=8) was also included in the experiment. Lambs of these ewes were weaned 18 days after lambing. No bacteria

were isolated from any teat catheter or mammary secretion samples obtained. These were used as inoculated negative controls. After selection, all animals were hand-milked thrice daily. Ewes were examined again and samples were collected as above, on the day of inoculation (D0), which was carried out as described by Mavrogianni et al. [20]. Ewes in A1, B1, C1 and D were challenged 2 mm deep into the teat by means of a sterile plastic fine catheter (Abbocath; Abbott Laboratories Inc., Abbott Park, IL, USA) 20 G. In the other teat of these ewes, 0.2 mL of sterile PBS was injected 2 mm deep into the teat. In ewes of subgroups A2, B2 and C2, 0.2 mL of sterile PBS was injected 2 mm deep into both teats. Ultimately, the teats of the ewes into each group were naturally infected (NI) and/or challenged (CH) as follows; subgroup A1, B1, C1: one teat NI+/CH+, the other teat NI-/CH-; subgroup A2, B2, C2: one teat NI+/CH-, the other teat NI-/CH-; group D: one teat NI-/CH+, the other teat NI-/CH-.

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II
Twelve, 3- to 5-years-old, Karagouniko-breed lactating ewes were included in the experiment. For selection, the same procedures and criteria as in Experiment I were applied. Allocation of animals into groups was carried out as follows; group E (n=6): isolation of coagulase-negative staphylococci in mild growth, group F (n=6): isolation of coagulase-negative staphylococci in mild growth. Additionally, a group G (n=6) containing ewes with no bacteria in the teat duct was included in the experiment; their selection was carried out as above and they were used as negative controls. After selection, the animals were hand-milked thrice daily. Then, the lower 3.0 to 3.5 cm of both teats of group E ewes or one teat of group G ewes were immersed into a 1 N solution of NaOH for 1 min; the procedure was repeated on the following day (D-1 and D0). The resulting chapping was scored according to the standards described by Fox et al. [7] and Mavrogianni et al. [22]. Ultimately, the teats of the ewes into each group were naturally infected (NI) and/or chapped (CP) as follows; group E: one teat NI+/CP+, the other teat NI-/CP+; group F: one teat NI+/CP-, the other teat NI-/CP-; group G: one teat NI-/CP+, the other teat NI-/CP-.

Post-inoculation/chapping examinations

After challenge or chapping, detailed clinical examination of the mammary glands and teats was carried out daily. Teat catheter samples and mammary secretion samples were collected. All samples were cultured onto Columbia blood agar; the media were incubated aerobically at 37 ^{o}C for up to 72 h. The CMT was carried out in secretion samples, as described before [11]. Secretion films were stained by the Giemsa method. Ewes were euthanized on sequential time-points. Dissection of the mammary glands and the teats started immediately; it was carried out as described before [20]. Scrapings from each of the two sites sampled in each teat, as well as parenchyma samples were examined by conventional bacteriological techniques [6]. Identification of staphylococci was carried by means of API-Staph SYSTEM quick identification strips (BioMerieux, Marcy-l'-Etoile, France) Conventional histopathological techniques were employed.

Data management and analysis

A scoring system previously developed and described [22] was used and numerical values were assigned for the pathological findings in the experimental animals. A separate score (0–4 scale)

was given for macroscopic and for histological findings in the teat and the mammary gland; these were then added to a 0–16 scale to produce a pathology score for the findings in each ewe.

Statistical analyses were performed in Minitab 14 (Minitab Inc., State College, PA, USA) and Epi-Info 6 (CDC, Atlanta, GA, USA). For analysis, the proportion of positive bacteriological and CMT results between the different groups / subgroups has been compared by using the Chi-square test or the Fisher Exact Test, as appropriate. Total pathology scores were compared using the Friedman Test using each day's total score as the unit and with group as "Treatment" and day number as "Block". Exact binomial Confidence Intervals (CI) for proportions were calculated. Statistical tests were 2-Sided.

RESULTS

Pre-inoculation/pre-chapping examinations

The mammary glands and the teats of all ewes were clinically healthy before challenge. The teats were soft with no external abnormalities. All selection criteria were fulfilled in the animals used. In Experiment I and Experiment II, bacteria recovered from teat duct catheter samples met the allocation criteria. In Experiment III, no bacteria were isolated from the mammary secretion or the teat duct catheter samples obtained.

Post-inoculation/post-chapping clinical, bacteriological and cytological findings

– Deposition of M. haemolytica into bacteriologically positive sheep teat ducts: Experiment I
None of the ewes in subgroup A1, B1 or C1 developed clinical mastitis. From the NI+/CH+ side, *M. haemolytica* was isolated: in total, from 16/32, 24/32, 25/32 samples from A1, B1, C1 ewes, respectively; additionally, the initial bacterial flora was also isolated from duct, but not from secretion, samples: in total, from 10/32, 14/32, 15/32 samples. The CMT increased (>"1"). None of the ewes in subgroup A2, B2 or C2 developed clinical or subclinical mastitis. From the NI+/CH- side, only the initial bacterial flora was isolated from duct, but not from secretion, samples: in total, from 16/32 samples from ewes of each subgroup. The CMT remained negative (<"1"). None of the ewes in group D developed clinical mastitis. From the NI-/CH+ side, *M. haemolytica* was isolated: in total, from 49/64 samples. The CMT increased (>"1"). No clinical signs were observed in any of the NI-/CH- sides (A, B, C, D ewes). No bacteria were recovered these. The CMT was negative. Details in Table 1.

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II
All ewes in group E developed systemic and mammary signs. The teats became chapped to score "2" to "3". *Staphylococcus* spp., same species as originally (before chapping) recovered from the teat duct catheter sample, were isolated in pure culture: in total, from 71/72 samples. The CMT increased (≥ "2"). Control teats (NI-/CP+) of ewes of group E remained chapped to a score "2" to "3"; no clinical findings characteristic of mastitis were observed. No bacteria were recovered. The CMT was mildly positive (score "1"). None of the ewes in group F developed clinical or subclinical mastitis. From the NI+/CP- side, only the initial bacterial flora was isolated from duct, but not from secretion, samples: in total, from 36/72 samples. The CMT remained negative (<"1"). The chapped teats of ewes of group G were scored "2" to "3". No mastitis was observed. From the NI-/CP+ side, no bacteria were recovered from any duct or secretion samples: from 0/72 samples. The CMT was positive (score "1"). No clinical signs were observed in any of the

NI-/CP- sides (F, G ewes). No bacteria were recovered. The CMT was negative. Details are in Table 1.

Table 1. Cumulative bacteriological findings and CMT results in samples after challenge of ewes during the three Experiments.

	Experiment I: subgroups						
	A1	A2	B1	B2	C1	C2	D
Bacterial isolation							
D-F[a]	10/16[b]	16/16	14/16	16/16	15/16	16/16	0/32
D-Mh[a]	10/16	0/16	14/16	0/16	15/16	0/16	29/32
S-F[a]	0/16	0/16	0/16	0/16	0/16	0/16	0/32
S-Mh[a]	6/16	0/16	10/16	0/16	10/16	0/16	20/32
CMT results							
Positive	14/16	0/16	14/16	0/16	14/16	0/16	28/32
	Experiment II: groups						
	E		F		G		
Bacterial isolation							
D-F[a]	36/36		36/36		0/36		
S-F[a]	35/36		0/36		0/36		
CMT results							
Positive	36/36		0/36		32/36		

[a] D-F = teat duct – flora, D-Mh = teat duct – *M. haemolytica*, S-F = secretion – flora, S-Mh = secretion – *M. haemolytica*, D-Ss = teat duct – *S. simulans*, S-Ss = secretion – *S. Simulans*
[b] n/m = positive results out of total animals sampled

Pathological Findings

– Deposition of M. haemolytica into bacteriologically positive sheep teat ducts: Experiment I
Post-mortem bacterial isolations were as follows. From the NI+/CH+ side of A1, B1 and C1 ewes, *M. haemolytica* was isolated: from 4/12, 10/12, 9/12 sites sampled, respectively (p=0.024); the initial bacterial flora was also isolated: from 3/12, 8/12, 8/12 sites sampled, respectively (p=0.062). From the NI+/CH- side of A2, B2 and C2 ewes, only the initial bacterial flora was isolated: from 7/12, 8/12, 7/12 sites sampled, respectively (p=0.89). From the NI-/CH+ side of D ewes, *M. haemolytica* was isolated in pure culture: from 15/24 sites sampled (0.625, 95% C.I.: 0.41–0.81). Statistical comparisons revealed that for A1 *vs* D, p=0.044, whilst for B1 or C1 *vs* D, p>0.4. No bacteria were isolated from the contralateral side (NI-/CH-) of these ewes. The total pathology scores summed over all days were 27, 33 and 35 for NI+/CH+ side of A1, B1 and C1 ewes (p=0.041), respectively (maximum possible: 64). The total pathology scores summed over all days were 8, 8 and 6 (p=0.37) for NI+/CH- side of A2, B2 and C2 ewes, respectively (maximum possible: 64). The median total pathology score summed over all days was 36 for NI-/CH+ side of D ewes (maximum possible: 64). Statistical comparisons revealed that for A1 *vs* D, p=0.038, whilst for B1 or C1 *vs* D, p>0.6.

– Artificial skin chapping on sheep teats with bacteriologically positive duct: Experiment II

Post-mortem bacterial isolations were as follows. From the NI+/CP+ side of E ewes, *Staphylococcus* sp. same species as originally recovered from the teat duct catheter sample, were consistently isolated in pure culture from the teat duct, teat cistern and mammary parenchyma: from 18/18 sites sampled; no bacteria were isolated from the contralateral side (NI-/CP-). From the NI+/CP- side of F ewes, *Staphylococcus* sp. same species as originally recovered from the teat duct catheter, were consistently isolated in pure culture from the teat duct: from 6/18 sites sampled; no bacteria were isolated from the other side (NI-/CP-). No bacteria were recovered either from the NI-/CP+ side of group G ewes: from 0/18 sites sampled or from their other side (NI-/CP-). The median total pathology score summed over all days was 28 for NI+/CP+ side of group E ewes (maximum possible: 32). The median total pathology score summed over all days was 3 for NI+/CP- side of group F ewes (maximum possible: 32). The median total pathology score summed over all days was 14 for NI-/CP+ side of group G ewes (maximum possible: 32). The median total pathology score summed over all days was 14 for NI-/CP+ side of group E ewes (maximum possible: 32). The median total pathology score summed over all days was 0 for NI-/CP- side of ewes of groups G and G (maximum possible: 32).

DISCUSSION

Previous experimental studies on ovine mastitis have established the protective role of the teat against intramammary infections; Mavrogianni et al. [20] studied the effects of the inoculation of *M. haemolytica* in different sites of healthy, bacteriologically negative teats. The results of that study showed that the ovine teat acted as a barrier against bacteria. During that study, we also suggested that the bacterial flora present in the teat duct of healthy ewes [10] might act competitively against invading bacteria and thus provide one of the defence mechanisms active in the teat.

In the present work, we inoculated bacteriologically positive teats with a *M. haemolytica* isolate, in order to study possible interactions between the bacterial flora and a confirmed mastitis causal agent [1, 2]. Ewes in subgroup A1 and D developed subclinical mastitis. However, recoveries of the challenge organism from the former animals were significantly fewer than from controls, thus suggesting an effect on the challenge strain; furthermore, the severity of the mammary lesions was significantly smaller in A1 than in D ewes. Adherence of *M. haemolytica* on mammary epithelial cells is required for its multiplication and leucotoxin production [32]; based on the present findings, one may postulate that the bacterial flora inhibited that process. No such bacteriological and pathological differences were seen in B1 and C1 ewes; this suggests that the protective effect of bacterial flora was exercised preferentially and only by staphylococcal species present in large numbers within the teat duct.

Bacterial competition is the situation where two bacterial populations compete for multiplication and survival, usually resulting in cell population reduction or impeded growth rate than if the two populations were separated [14]. This was evident in subgroup A1, where a distinct protective effect of the flora was recorded. Both the flora populations and the challenge, invading organism were subsequently recovered from a reduced number of samples than from respective controls. This type of relationship between bacteria occurs when two species compete to occupy a particular site [18, 31]. Rainard and Poutrel [27] have also reported that new infections were less frequent in glands already harbouring a pathogen. All these findings further support the above hypothesis.

Production of antagonistic substances by bacterial flora and competition for necessary nutritional substances between flora and invading organisms [30] are also contributing mechanisms. The direct toxic effects of certain bacterial species against other ones invading the host have also been considered, as flora populations can secure their domination over invading pathogens by producing antibacterial substances [4, 15]. Staphylococcal strains isolated from cows' teat orifice or mammary secretion, have been found to produce bacteriocins and reduce in vitro growth of other pathogens [5, 25].

However, when the microbial equilibrium is disrupted for any reason, it is possible that pathogenicity of the flora strains would increase, leading to disease. Mayrand and Grenier [24] studied bacterial interactions and found that once the intra-bacterial balance was broken, pathological changes were initiated. Under those circumstances, the flora would contribute to development of disease either by facilitating an invader to fully expressing its pathogenicity or even by participating in the infectious process itself in order to establish the pathological findings. The findings of Experiment II clearly indicate that under certain circumstances, the resident bacterial flora can become pathogenic. Ewes in group E rapidly developed acute clinical mastitis without bacterial challenge; the disease was caused by the bacterial flora organisms, which multiplied and ascended to the mammary parenchyma. Lesions observed during this study were typical of staphylococcal mastitis [8]. In this case, the “trigger factor” that led to the equilibrium shift was the teat chapping.

In a recent paper, Mavrogianni et al. [22] provided evidence that teat chapping predisposed ewes to mastitis in cases of new bacterial infections. Chapped teats are considered an increased risk for mastitis [26, 28]. During cold weather, increased incidence of chapped teats has been reported [7]. In ewes, Leyshon [17] has reported that mastitis was more prevalent in cold weather; this could have been the consequence of chapped teats.

In damaged tissues there is reduced responsiveness and defective chemotaxis of neutrophils [3], which cannot withstand the low pH and high temperature in chapped tissues [12, 16]. Additionally, the reduced hydration of chapped skin alters skin microflora, consequently decreasing resistance to bacterial colonization. We thus believe that in these circumstances, depletion of cellular defences consequently to chapping, resulted in shifting of the balance and allowed bacterial invasion and mastitis. One may also suggest that exposure to trauma may cause degranulation and lysis of mast cells, which are active during acute stages of inflammation [13], consequently reducing the defence abilities of the teat.

Perhaps under field conditions and on a longer-term basis, any factors affecting the immune status of the animals, would affect the equilibrium of flora organisms within the teat, thus resulting to mastitis.

In the past, presence of bacterial flora within a mammary gland has been advocated as a means of preventing mastitis in cows [19]. From that viewpoint, preservation of a protective teat duct flora would be useful for prevention of the disease. Nevertheless, an intramammary infection with a microorganism, even in small doses, might result in increased somatic cell counts, tissue damage and adverse production effects [29]. On the other hand also, teats harbouring bacteria can be a source of infection for the mammary gland. Any impediment of the defence mechanisms (local or systemic) may shift the balance and allow the bacteria to multiply, invade the mammary gland and cause mastitis.

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REFERENCES

[1] Bergonier D., Berthelot X., New advances in epizootiology and control of ewe mastitis, Livest. Prod. Sci. 79 (2003) 1–16.
[2] Bergonier D., De Cremoux R., Rupp R., Lagriffoul G., Berthelot X., Mastitis of dairy small ruminants, Vet. Res. 34 (2003) 689–716.
[3] Cheville N.F., Ultrastructural Pathology: an Introduction to Interpretation, Iowa State University Press, Ames, 1994.
[4] Daw M.A., Falkiner F.R., Bacteriocins: nature, function and structure, Micron 27 (1996) 467–479.
[5] De Vliegher S., Opsomer G., Vanrolleghem A., Devriese L.A., Sampimon O.C., Sol J., Barkema H.W., Haesebrouck F., de Kruif A., In vitro growth inhibition of major mastitis pathogens by Staphylococcus chromogenes originating from teat apices of dairy heifers, Vet. Microbiol. 101 (2004) 215–221.
[6] Euzeby J.P., List of bacterial names with standing in nomenclature: a folder available on the internet, Int. J. Syst. Bacteriol. 47 (1997) 590–592. (List of Prokaryotic Names with Standing in Nomenclature. Last full update September 07, 2006. URL: http://www.bacterio.net).
[7] Fox L.K., Nagy J.A., Hillers J.K., Cronrath J.D., Ratkowsky D.A., Effects of postmilking teat treatment on the colonization of Staphylococcus aureus on chapped teat skin, Am. J. Vet. Res. 52 (1991) 799–802.
[8] Fthenakis G.C., Jones J.E.T., The effect of inoculation of coagulase-negative staphylococci into the ovine mammary gland, J. Comp. Pathol. 102 (1990) 211–219.
[9] Fthenakis G.C., Prevalence and aetiology of subclinical mastitis in ewes of Southern Greece, Small Rumin. Res. 13 (1994) 293–300.
[10] Fthenakis G.C., Marples R.R., Richardson J.F., Jones J.E.T., Some properties of coagulase-negative staphylococci isolated from cases of ovine mastitis, Epidemiol. Infect. 112 (1994) 171–176.
[11] Fthenakis G.C., California Mastitis Test and Whiteside Test in diagnosis of subclinical mastitis of dairy ewes, Small Rumin. Res. 16 (1995) 271–276.
[12] Harlan J.M., Schwartz B.R., Reidy M.A., Schwartz S.M., Ochs H.D., Harker L.A., Activated neutrophils disrupt endothelial monolayer integrity by an oxygen radical-independent mechanism, Lab. Invest. 52 (1985) 141–150.
[13] Henderson W.R., Chi E.Y., Klebanoff S.J., Eosinophil peroxidase-induced mast cell secretion, J. Exp. Med. 152 (1980) 265–279.
[14] Isenberg H.D., D'Amato R.F., Indigenous and pathogenic microorganisms of humans, in Lennette E.H., Balows H., Hausler W.J. Jr, Shadomy H.J. (Eds), Manual of clinical microbiology, American Society for Microbiology, Washington, 1985, pp. 24–35.
[15] Jack R.W., Tagg J.R., Ray B., Bacteriocins of gram-positive bacteria, Microbiol. Rev. 59 (1995) 171–200.
[16] Jacques Y.V., Bainton D.F., Changes in pH within the phagocytic vacuoles of human neutrophils and monocytes, Lab. Invest. 39 (1978) 179–185.
[17] Leyshon W.J., An examination of a number of cases of ovine mastitis, Vet. J. 85 (1929) 286–300 & 331–344.
[18] Lina G., Boutite F., Tristan A., Bes M., Etienne J., Vandenesch F., Bacterial competition for human nasal cavity colonization: role of staphylococcal agr alleles, Appl. Environ. Microbiol. 69 (2003) 18–23.
[19] Martin G., Bergmann A., Effect of targeted bacterial colonization of cattle udder on subsequent on subsequent infection by pathogenic germs, Monatsh. VeterinarMed. 46 (1991) 770–773.

[20] Mavrogianni V.S., Fthenakis G.C., Brooks H., Papaioannou N., Cripps P.J., Taitzoglou I., Brellou G., Saratsis P., The effects of inoculation of Mannheimia haemolytica into the teat of lactating ewes, Vet. Res. 36 (2005) 13–25.
[21] Mavrogianni V.S., Cripps P.J., Fthenakis G.C., Description and validation of a novel technique to study the bacterial flora of the teat duct of ewes, Small Rumin Res. 66 (2006) 258–264.
[22] Mavrogianni V.S., Cripps P.J., Papaioannou N., Taitzoglou I, Fthenakis G.C., Teat disorders predispose ewes to mastitis after challenge with Mannheimia haemolytica, Vet. Res. 37 (2006) 89–105.
[23] Mavrogianni V.S., Cripps P.J., Brooks H., Taitzoglou I.A., Fthenakis G.C., Presence of sub-epithelial lymphoid nodules in the teat of ewes, Anat. Histol. Embryol. (2007) doi:10.1111/j.1439-0264.2006.00720.x.
[24] Mayrand D., Grenier D., Bacterial interactions in periodontal disease, Bul. Inst. Pasteur 96 (1998) 125–133.
[25] Nascimento J.D.S., Fagundes P.C., Brito M.A.V.D., dos Santos K.R.N., Bastos M.D.D., Production of bacteriocins by coagulase-negative staphylococci involved in bovine mastitis, Vet. Microbiol. 106 (2005) 61–71.
[26] Neave F.K., Dodd F.H., Kingwill R.G., Westgarth D.R., Control of mastitis in the dairy herd by hygiene and management, J. Dairy Sci. 52 (1969) 696–706.
[27] Rainard P., Poutrel B., Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle, Am. J. Vet. Res. 49 (1988) 327–329.
[28] Rainard P., Riollet C., Innate immunity of the bovine mammary gland, Vet. Res. 37 (2006) 369–400.
[29] Saratsis Ph., Alexopoulos C., Tzora A., Fthenakis G.C., The effect of experimentally induced subclinical mastitis on the milk yield of dairy ewes, Small Rumin. Res. 32 (1999) 205–209.
[30] Smith H., The revival of interest in mechanisms of bacterial pathogenicity, Biol. Rev. 70 (1995) 277–316.
[31] Tsuno H., Hidaka T., Nishimura F., A simple biofilm model of bacterial competition for attached surface, Water Res. 36 (2002) 996–1006.
[32] Vilela C.L., Fitzpatrick J., Morgan K.L., In vitro adherence and invasion of ovine mammary epithelium by Mannheimia haemolytica, Vet. J. 167 (2004) 211–213.