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DOI: 10.2478/fv-2018-0028

FOLIA VETERINARIA, 62, 3: 56—61, 2018

MASTITIS PATHOGENS ISOLATED FROM RAW MILK SAMPLES ON SHEEP FARMS SITUATED IN MARGINAL PARTS OF SLOVAKIA

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ABSTRACT

Relatively large parts of the Slovak territory are marginal regions, which in terms of the economy of ruminant rearing can effectively produce animal commodities only occasionally. The aim of this study was to evaluate the occurrence and aetiology of mastitis during one milking season in two sheep dairy herds of 224 (A) and 270 (B) ewes situated in the marginal parts of Slovakia. A complex examination of health status of ewes based on: the clinical examination of the udder, macroscopic evaluation of milk with the California mastitis test (CMT) and the bacteriological analysis of raw milk samples from individual halves were carried out at the beginning (April) and at the end (September) of the milking season. The prevalence of intramammary infection (IMI) in the herds of ewes (A and B) were 19.1 % and 14.3 %, respectively. In both herds we confirmed predominantly the subclinical forms of IMI. The highest percentages of aetiological agents were determined for coagulase negative staphylococci (CoNS), such as *Staphylococcus chromogenes*, *Staphylococcus schleiferi*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Staphylococcus piscifermentans* and *Staphylococcus intermedius*, the occurrences of which were determined in 41 (48.2 %) and 37 (47.9 %) positive milk samples, respectively.

Key words: coagulase negative staphylococci; ewes; marginal parts; mastitis; prevalence

INTRODUCTION

A problem involving the agricultural production in marginal areas exists not only in highly developed countries but is typical also for countries in the transient period of economy development. Slovakia is one of these countries, with a relatively high share of marginal agricultural areas [15]. Products from dairy ruminants are unique, especially in the field of rational nutrition of consumers. Many of these products and specialties can be included among the functional foods [20].

The production of sheep milk is currently the main aim of many agricultural farms localized in marginal regions.

Milk plays a crucial role in the economy of cooperatives and farms. The price of milk is affected by sheep milk market, but also by farmers themselves. On the farmer's side, there are legislative limits for the total number of microorganisms in the milk delivered, which cannot be exceeded. An equally important contribution of breeders is also the hygienic safety of the milk for the consumers, especially in the marketing of milk and milk products directly sold on the farm [7, 17].

Mastitis is an important disease of ewes occurring in all countries of the world where sheep are kept. This inflammation of the mammary gland is known to be a complex and costly disease [1, 16]. The disease is associated with a decrease in milk production, an increase of veterinary services, treatment, labour costs and culling [3, 14].

Sheep mastitis is predisposed by several epidemiological risk factors that play a significant role in causing mammary incompetence to protect it from the invasion of infectious agents. Many bacterial agents, such as *Staphylococcus aureus, Streptococcus agalactiae, E. coli* or coagulase negative staphylococci (CoNS) have been found to be associated with clinical (Fig. 1) or subclinical mastitis in ewes [2, 7].

The aim of this study was to evaluate the occurrence and aetiology of mastitis at the beginning (April) and the end (September) of the milking season in two dairy sheep herds situated in marginal parts of Slovakia.

MATERIALS AND METHODS

Animals and milking

The practical part of the study was performed on two sheep herds situated in marginal parts of eastern Slovakia (Gelnica, Trebišov), kept under standard animal husbandry and hygiene conditions. The herd A (224 sheep) consisted of the breeds, Improved Valaska and Lacaune. The herd B (270 sheep) consisted of the breeds, Improved Valaska and Tsigaia. The ewes were milked twice a day after weaning of their lambs at the beginning of April. In herd A, machine milking was performed using two-line milk parlour 2 × 14 Miele Melktechnik, (Hochreiter Landtechnik, Germany) and in herd B sheep were milked in two-line milk parlour 2 × 15 Alfa Laval Agri (Alfa Laval, Sweden).

Collection of samples and laboratory analyses

The complex examination of the health status of the udders in ewes was carried out at the beginning (April) and the end (September) of the milking season. The sheep were examined clinically according to Hariharan et al. [5], e. g. for swelling, presence of lesions or anatomical malformations, and milk from individual halves were evaluated by the California mastitis test (CMT). The CMT scores were 0, +, ++, and +++ for: "negative", "weak positive", "positive", and "strong positive", respectively [3]. The emphasis was put on aseptic sampling and transport of the milk samples from individual halves intended for bacteriological examination.

The bacteriological examinations were performed according to the commonly accepted rules [11]. Milk samples (0.05 ml) were inoculated onto blood agar (Oxoid, UK) and cultivated at 37 °C for 24 hours. Based on the colony morphology and Gram staining, bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, Slovakia). The suspected colonies of *Staphylococ-*

Fig. 1. Peracute mastitis caused by *Staphylococcus aureus*

cus spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar, cultivated at 37 °C for 24 hours and identified biochemically using the STAPHY-test, STREPTO-test, or ENTERO-test and identified by software TNW Pro 7.0 (Erba-Lachema, Czechia).

The health of the udder and individual forms of mastitis (subclinical and clinical) based on clinical signs, abnormal udder secretions, CMT scores and bacteriological examination with positive culture result were classified according to Fthenakis [3, 4].

Statistical analysis

The results were analysed statistically using the GraphPad Prism 6.0 (GraphPad Software Inc., USA). The differences in the prevalence of mastitis among herds were statistically analysed using the Chi-square test. The level of significance was set to $P < 0.05$.

RESULTS

Table 1 shows the prevalence of intramammary infection (IMI) in dairy sheep herds during the milking season. The evaluation of the CMT showed that 23.2 % of the samples in herd A and 16.6 % of the samples in herd B were scored as either weak positive, positive or strong positive. In herd B, we observed a decreased prevalence of positive halves as well as a higher number of healthy halves. The prevalence of IMI with the positive CMT and bacteriological cultivation of individual raw milk samples in the monitored herds A and B of ewes was 19.1 % to 14.3 %, respectively.

The prevalence of subclinical mastitis among herds A and B varied from 13.9 % to 6.5 %, respectively. The differences in the prevalence of subclinical forms in the examined halves among herds were significant at the beginning of milking season ($P < 0.05$) (Fig. 2). The prevalence of clinical mastitis ranged from 1.8 % to 2.2 %.

The bacteria isolated from the infected halves of the herds are presented in Tables 2 and 3. Pathogenic bacteria were isolated from 16.5 % (162) of all of the 981 investigated halves. The most frequent aetiological agents of mastitis were CoNS, *Staphylococcus aureus, Streptococcus sanguinis, Streptococcus uberis* and *Enterococcus* spp. Staphylococci were the main aetiological IMI agents in the dairy sheep herds. In both herds CoNS occurred in the highest proportion (A: 41, [48.2 %]; B 37, [47.9 %]) in the positive cases. The bacteria of the genus *Staphylococcus* were the most numerous and besides *Staphylococcus aureus* there were isolated also *Staphylococcus chromogenes, Staphylococcus schleiferi, Staphylococcus piscifermentans, Staphylococcus epidermidis, Staphylococcus intermedius,* and *Staphylococcus xylosus*. Of these, CoNS, *Staphylococcus chromogenes* was isolated regularly throughout the milking season in the breeds with clinical IMI in two cases. S*taphylococcus aureus* with *Streptococcus uberis* and *Streptococcus sanguinis* were the most frequent isolates from the clinical mastitis cases.

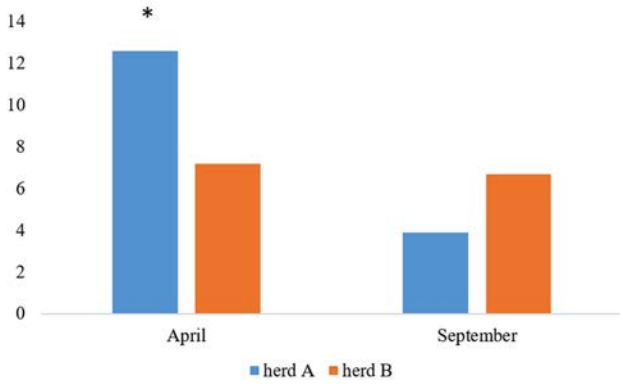

Fig. 2. Comparison of subclinical forms of mastitis [%] during the milking season
*P < 0.05 — significant differences in the incidence of subclinical mastitis among herds

Table 1. Prevalence of mastitis in the examined halves of dairy sheep herds

Herd	No. of examined sheep	No. of examined halves	Healthy halves		Rejected halves	Positive halves*		Infected halves	
			n	%		n	%	n	%
A	224	444	341	76.8[a]	4	103	23.2[a]	85	19.1
B	270	537	448	83.4[b]	3	89	16.6[b]	77	14.3
Total	**494**	**981**	**789**	**80.4**	**7**	**192**	**19.6**	**162**	**16.5**

* — CMT with score +, ++ and +++; [a, b] — values within the same column with different superscript letters differ significantly at $P < 0.05$

Table 2. Bacteria isolated from positive halves of herd A

Bacteria			Subclinical forms				Clinical forms			
			April		September		April		September	
	n	%	n	%	n	%	n	%	n	%
S. aureus	9	10.6	4	4.6	1	1.1	3	3.4	1	1.1
S. chromogenes	15	17.2	11	12.6	3	3.4	–	–	1	1.1
S. piscifermentas	11	12.6	9	10.6	2	2.3	–	–	–	–
S. schleiferi	10	11.5	7	8.0	3	3.4	–	–	–	–
S. xylosus	5	5.7	5	5.7	–	–	–	–	–	–
Str. sanguinis	8	9.2	4	4.6	2	2.3	1	1.1	1	1.1
Ent. faecalis	14	16.1	10	11.5	3	3.4	1	1.1	–	–
E. coli	7	8.0	2	2.3	5	5.7	–	–	–	–
Other*	6	6.9	4	4.6	2	2.3	–	–	–	–
Total	**85**	**100**	**56**	**64.3**	**21**	**24.1**	**5**	**5.7**	**3**	**3.4**

n — number of isolated bacteria; other* — *Proteus* spp., *Aerococcus* spp.

Table 2. Bacteria isolated from positive halves of herd B

Bacteria			Subclinical forms				Clinical forms			
			April		September		April		September	
	n	%	n	%	n	%	n	%	n	%
S. aureus	17	22.1	6	7.8	7	9.1	1	1.3	3	3.9
S. intermedius	8	10.4	1	1.3	7	9.1	–	–	–	–
S. epidermidis	11	14.3	4	5.2	7	9.1	–	–	–	–
S. chromogenes	11	14.3	3	3.9	6	7.8	1	1.3	1	1.3
S. schleiferi	8	10.4	5	6.5	3	3.9	–	–	–	–
Str. uberis	7	9.1	2	2.6	2	2.6	1	1.3	2	2.6
Str. sanguinis	2	2.6	1	1.3	–	–	1	1.3	1	1.3
Ent. faecalis	2	2.6	2	2.6	–	–	–	–	–	–
Ent. gallinarum	2	2.6	2	2.6	–	–	–	–	–	–
E. coli	4	5.2	4	5.2	–	–	–	–	–	–
Other*	5	6.5	2	2.6	3	3.9	–	–	–	–
Total	**77**	**100**	**32**	**41.6**	**35**	**45.8**	**4**	**5.2**	**7**	**8.8**

n — number of isolated bacteria; other* — *Proteus* spp., *Bacillus* spp.

DISCUSSION

The prevalence rate of mastitis in sheep flocks usually varies from 1 to 30 %. In our study the prevalence of mastitis in the A and B herds of dairy ewes was 19.1 % and 14.3 %, respectively. In the study by Contreras et al. [2], in only 10 % of the flocks had a prevalence that exceeded 10 %. In fact, within a flock the prevalence risk of mastitis exceeding 20 % is very rare; as is bilateral mastitis caused by common mammary pathogens.

Subclinical mastitis is financially the most crippling form of the disease [19] because it has detrimental effects on the milk yield of ewes and on the growth of their lambs [8]. At the start and end of the lactation season we confirmed predominantly subclinical forms in both herds. The economic losses are more associated with subclinical mastitis which is 40 % more prevalent than clinical mastitis [6]. The clinical cases were most frequently caused by *Staphylococcus aureus*, *Str. uberis* and *Str. sanguinis*.

Many microbial species that are common causes of ruminant mastitis, such as *Streptococcus agalactiae, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*, also occur as commensals or pathogens of humans whereas other causative species, such as *Streptococcus uberis, Streptococcus dysgalactiae* subsp. dysgalactiae or *Staphylococcus chromogenes*, are almost exclusively found in animals [9, 21].

Although a wide range of microorganism species may cause sheep mastitis, most cases are reported to be due to staphylococci [12].

Lafi and Hailat [10] reported that *S. aureus* was the predominant bacterial strain (50 %) in milk from ewes, followed by *E. coli* (27 %) and *Pseudomonas aeruginosa* (7 %). Narenji et al. [13] reported that *Staphylococcus aureus* (72.2 %) and CoNS (66.6 %) were the most common isolates from dairy ewes' subclinical mastitis. The main *Staphylococcus aureus r*eservoirs in sheep are suggested to be the infected mammary glands and teat lesions. However, *Staphylococcus aureus* can also be cultured from intact teat skin and other body sites.

The importance of coagulase negative staphylococci in the aetiology of sheep mastitis was also confirmed in our study when six species isolated from subclinical mastitis were recorded in the 79 individual samples from the two sheep breeds. Only *Staphylococcus chromogenes* together with the coagulase positive *S. aureus* caused the clinical forms of mastitis.

Thorberg et al. [18] in their study isolated *Staphylococcus epidermidis* and *Staphylococcus chromogenes* from the raw milk of dairy ruminants. The same bacteria were also isolated from skin of the people that milked the dairy animals, because isolation of *Staphylococcus epidermidis* from human skin is more common than isolation from skin of the udder. The authors concluded that humans are probably the main source of infection with coagulase negative species during milking.

CONCLUSIONS

In conclusion, the results of this study indicated that the prevalence of mastitis in the herds A and B reached 19.1 % and 14.3 %, respectively. It was also observed that CoNS are the most common cause of subclinical mastitis in dairy sheep herds situated in the marginal parts of Slovakia.

A successful mastitis control program should focus on good management that can ensure: a clean and comfortable environment, proper feeding and adequate supplementation of the diet with vitamins and trace elements. The prevention of mastitis consists in a reduction of the exposure of the teat ends of the mammary gland to the environmental pathogenic bacteria capable of surviving in the external environment outside the udder.

ACKNOWLEDGEMENT

This study was supported by the project VEGA No. 1/0510/16.

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Received July 19, 2018
Accepted August 31, 2018