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Original Paper

Sheep mastitis caused by staphylococci and streptococci and their influence on oxidative status

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The objectives of this study were to determine the relationship of oxidative product levels, using malondialdehyde (MDA) as a marker on occurrence of mastitis and its causing pathogens in two dairy flocks of ewes situated in east and north of Slovakia. The diagnosis of mastitis was performed on the basis of clinical examination of the udder, macroscopic evaluation of milk, with the evaluation of Californian mastitis test (CMT) and bacteriological analysis of individual raw milk samples. From total 537 and 444 halves ewe's milk samples were 16.6% and 23.2% positive to CMT, respectively. The prevalence of mastitis caused by bacterial pathogenes in the monitored herds was 14.3% to 19.1%, respectively. In all monitored sheep flocks were confirmed predominantly subclinical forms (SM) of intramammary infection (IMI). The highest of etiological agents in all monitored herds had coagulase negative staphylococci and coagulase positive staphylococci especially *Staphylococcus aureus*. Except for staphylococci were *Streptococcus uberis* and *Streptococcus* spp. most frequently pathogens isolated from mastitic sheep. The highest MDA level was observed from clinical cases of mastitis however, increased MDA levels were detectable from subclinical cases. Bacterial isolates from mastitc halves milk samples are different in levels of MDA. In this study, we found that milk samples infected with *S. aureus* were higher compared to other pathogens. In conclusion, differences in both severities of IMI and mastitis pathogens were associated with differences of MDA in infected udders.

Keywords: sheep, milking, mastitis, lipid peroxidation, *S. aureus*, coagulase negative staphylococci

1 Introduction

One of the major factors that negatively affect the quality and production of milk is inflammation of mammary gland – mastitis. Mastitis are nowadays both economic and health problems. They reduce not only the quality of milk production but can also lead to the removal of the animals from the breeding. Poor hygienic, biological, and nutritional parameters often lead to the development of mastitis (Contreras et al., 2007).

Inflammation of the mammary gland causes a change in milk composition and its quality as well as leads to a reduction in milk production. It is most often of bacterial origin, where pathogenic species of microorganisms such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, coagulase negative staphylococci (CNS), *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* can be found. Milk may also contain inhibitory substances, such as antibiotics or drugs that can cause microorganism resistance, which is severally related to treatment of the population. Mastitis has different causes and symptoms, varying degrees of intensity, different durations and different resultant effects (Mørket et al., 2007; Pyörälä and Taponen, 2009).

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Increase of oxidative stress in dairy sheep as a consequence of inflammation mammary gland can result in excess accumulation of reactive oxygen species (ROS), which can induce lipid peroxidation and reduction of antioxidative activity due to catalysis of various hydrogen and lipid peroxides. Oxidative stress in veterinary medicine and particularly in ruminant health is a relatively young field of research (Sharma, 2011). Detection of malondialdehyde (MDA) from milk samples may be helpful in the health management of ewes. Therefore, the goal of this study was to determine the changes in levels of oxidative product, using MDA as a marker on severity of mastitis.

2 Material and methods

2.1 Farms and milking

The practical part of the study was carried out in two dairy herds of ewes situated in east and north of Slovakia.The first herd situated in east of Slovakia consists of 370 sheep of the breeds Lacaune, Improved Wallachian and their crosses. In the months of April to October, they are moved to pasture where there is a rich and diverse grassland. In the winter, sheep are housed in sheep – stall on deep litter. On this farm, ewes are milked twice a day until the end of summer season in the early morning and late afternoon after coming from the pastures. With approaching decreasing milk production and approaching drying time, the milking frequency is reduced to once a day. On the farm there is an Alfa Laval Agri 2 × 20 machine milking parlor, where are the aisles, a staff room, a milk cooling and storage room and a waiting room.

Figure 1 Waiting room and machine milking parlor on the first farm

Figure 2 Mobile machine parlor and milking sheep on the second farm

The second farm is situated in north of Slovakia with capacity 400 sheep of breeds Lacaune, Improved Wallachian and Slovak dairy sheep. At the beginning of the pasture season and in the summer period approximately until the end of August, the milking frequency was twice a day in the morning and evening after returning from the pasture. At the end of August, September and approximately by October, milking was once a day and the sheep will prepare to dry. During all milking season the ewes are milked outside, with the help of a mobile machine parlor Farmtec (DOK 2x12). It is an in – line milking parlor with 2 × 12 milking stands between which there is a food tray (Figure 2). Before milking, all the sheep are driven into the enclosed space in front of the milking parlor then proceed up a small ramp where they are secured.

2.2 Examination and sampling

The complex examination of the health status of the udders in ewes from both herds 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. (2004) 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 (Fthenakis, 1995). Two samples of 10 ml of freshly milked milk were collected aseptically and placed into sterile tubes. The samples were immediately placed into a refrigerated container with a temperature of 5 °C. In the laboratory were samples diagnosed by cultivation and isolation of bacterial agents causing mastitis of sheep according to commonly accepted rules (Malinowski et al., 2008). After cultivation and detection of virulence factors were bacteria identified biochemically using the STAPHY-test, STREPTO-test, resp. ENTERO-test and identification by software TNW Pro 7.0 (Erba-Lachema, CZ) with precision of detection over 90.0%.

2.3 Groups selection for MDA detection

Of the both herds for milk MDA detection during first and second complex examination were selected 32 ewes (64 halves milk samples) on the basis positive CMT score and bacteriology cultivation with subclinical mastitis (SM), which they didn't show clinical signs of mastitis or other illnesses as well as 10 sheep (20 halves milk samples) with clinical mastitis and high score of CMT or clinical signs. As control were selected 10 healthy sheep (20 halves milk samples) without clinical signs and negative score of CMT. Samples were immediately placed in crushed ice and submitted to the laboratory within 2–4 hrs.The MDA level from selected milk samples was measured by the photometric method based on a reaction with thiobarbituric acid (TBA) as described by Suriyasathaporn et al. (2006).

2.4 Statistical analyses

The differences in the prevalence of mastitis among herds were statistically analysed using the Chi-square test. The data of milk MDA level from selected groups of sheep and selected mastitis pathogens are presented as the mean (*M*) ± standard deviation (*SD*). Difference between groups and pathogens causing mastitis were analysed by using analysis of variance (ANOVA) followed by Tukey comparison test and minimum criteria for statistical significance was set at $p \leq 0.05$ for all. Approximate probabilities were evaluated with Post Hoc Test for the statistical analysis of MDA level between selected pathogens.

3 Results and discussion

Table 1 shows the prevalence of intramammary infection (IMI) in monitored dairy sheep herds during the milking season.The evaluation of the CMT showed that 16.6% of the samples in herd A and 23.2% of the samples in herd B were scored as either weak positive, positive or strong positive. In herd A, 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 14.3% to 19.1%, respectively. Bacteria *Staphylococcus aureus* with *Streptococcus uberis* and *Streptococcus sanguinis* were the most frequent isolates from the clinical mastitis cases. During the first investigation (April), in both herds CNS occurred in the highest proportion (41.6% and 64.3%) in the SM cases. After second investigation (September) were CNS occurred of 45.8% and 24.1% in the SM cases.

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	270	537	448	83.4[b]	3	89	16.6[b]	77	14.3
B	224	444	341	76.8[a]	4	103	23.2[a]	85	19.1
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 $P < 0.05$

In the mammary gland, phagocytic cells migrate to the place of inflammation and, depending on their activity, use more oxygen, resulting in end stage lipid peroxidation. Thus, the level of MDA increases (Turk et al., 2017). Averages of milk MDA for the selected groups and for the selected pathogens causing subclinical and clinical mastitis were shown in Figure 3 and Table 2.

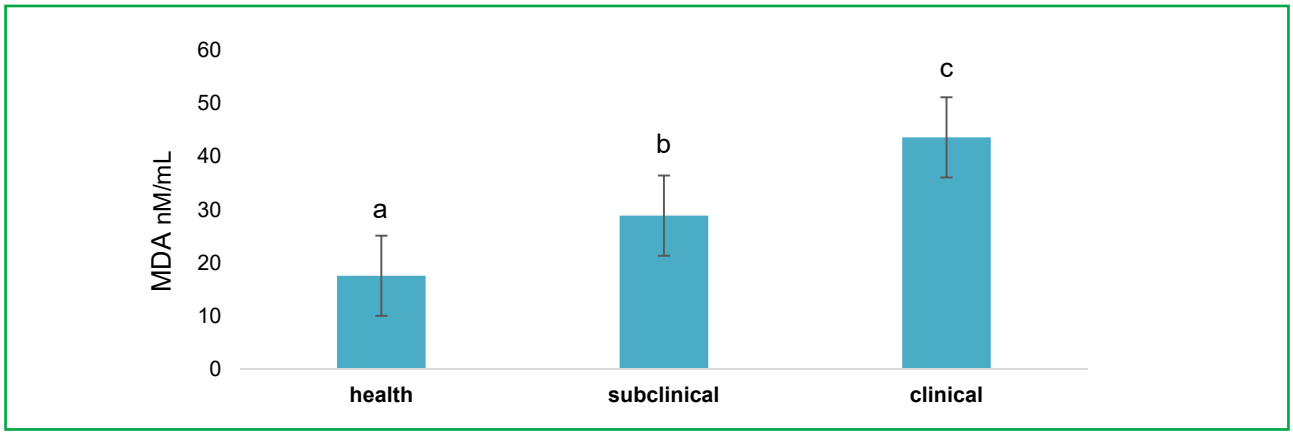

Figure 3 Milk malondialdehyde level from selected ewe's milk samples
MDA concentration separated by healthy halves ($n = 20$), 32 halves with subclinical mastitis ($n = 64$) and 10 halves with clinical mastitis ($n = 20$), a,b,cOverall MDA values without common superscript differ significantly at $P < 0.05$

Average, values of milk MDA in this study from health, subclinical and clinical group were 17.6, 28.9, and 59.6 nM/ml, respectively. From univariate analyses, results show that differences between selected groups and pathogens causing SM and CM were related to MDA concentrations ($p < 0.05$). Milk from SM and CM quarters had higher MDA concentrations, and were statistically different ($p < 0.05$) compared to healthy quarters (Figure 3). According to our expectation, CM halves had highest MDA concentrations. This might be caused by the higher levels of udder defense mechanism. With the perspective of early diagnosis have shown increased MDA levels in SM halves. This study showed that MDA level was different among pathogens causing IMI (Table 2). Characteristics of pathogens may be the reason for differences in oxidative environment in udders (Suriyasathaporn et al., 2006). Results from Post Hoc probabilities showed, that MDA from milk samples infected with *S. aureus* were statistically higher ($p < 0.001$) compared to other pathogens.

Table 2 Comparison of MDA concentrations between milk samples and individual bacterial pathogens

Groups	Normal 21.40	*S. chromogenes* 27.34	*E. coli* 29.72	*Str. sanguinis* 30.85	*S. aureus* 57.71
Normal		0.000159	0.000134	0.000134	0.000134
S. chromogenes	0.000159		0.206953	0.019734	0.000134
E. coli	0.000134	0.206953		0.019734	0.000134
Str. sanguinis	0.000134	0.019734	0.019734		0.206953
S. aureus	0.000134	0.000134	0.000134	0.206953	

Tukey HSD test; variable MDA approximate probabilities for Post Hoc Tests Error: Between MS = 5.9733, *df* = 45.000

4 Conclusions

We confirmed that the current pathogens mammary gland includes CNS, *S. aureus*, *Str. sanguinis* and *E. coli*, which were most often isolated from SM and CM. It was also observed that CNS were the most common cause of SM in monitored dairy sheep herds. The highest MDA level was observed from clinical cases of mastitis however; elevated in levels of MDA were detectable from SM halves. Bacterial isolates from subclinical halves milk samples are different levels of MDA. *S. aureus* isolated from mastitic milk samples was statistically higher compared to other pathogens. Subclinical mastitis is difficult to detect because of a lack of clinical signs that can be easily identified by visual inspection and palpation of the udder. As can be seen from our study, one of the additional methods for detecting SM can be measurement of MDA level in raw sheep's milk.

Acknowledgments

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