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MASTITIS PATHOGENS ISOLATED FROM SAMPLES OF MILK IN DAIRY SHEEP AND THEIR RESISTANCE

Veterinary Medicine

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ABSTRACT

The objective of this study was to evaluate the effectiveness of different antibiotics against mastitis causing microorganisms in lactating dairy sheep in farm of east Slovakia. Milk samples from individual halves were cultured and bacteriologically evaluated. All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to a large number of antibiotics. The results revealed higher sensitivity against Amoxicillin (100% Coagulase Negative Staphylococci (CNS)). Medium level of resistance was observed against ampicillin (10.7%), cloxacillin (11.7%), oxacillin (10.7%) and penicillin (11.7%).

KEYWORDS:

dairy sheep, mastitis, resistance, coagulase negative staphylococci

1 Introduction

Mastitis is one of the most economically important diseases of dairy animals. It causes great economic losses and affects the quality and quantity of milk. Mastitis generally results from interaction between a variety of microbial infections and host responses in the udder [1].

The occurrence of intramammary infections (IMI) in sheep is in interval from 4.0 to 50.0 %. The annual incidence of clinical IMI in dairy sheep is generally lower than 5 %, but in a small percentage of herds the incidence may exceed 30–50 % of the animals, causing mortality (gangrenous mastitis) or culling of up to 70 % of the herd [2,3,4].

The predominant contagious pathogens involved in sheep and goat mastitis are Coagulase negative staphylococcoci (CNS), *Staphylococcus aureus*, *Streptococcus uberis*, *Strep. dysgalactiae*, and *Strep. agalactiae*. Environmental pathogens are transmitted during milking from the environment serving as the primary source of these organisms. The main pathogens in this group are *Strep. equinus*, *Strep. mitis*, *Strep. salivarius*, *Strep. saccharolyticus*, *Pasteurella sp., E. coli*, *Enterococcus faecalis*, *E. faecium*, *E. avium*, and *Aerococcus viridans* [5].
However, these organisms have a profound importance in both human and veterinary medicine. Antibiotic treatment of mastitis leads to significant increase in milk quantity and quality, lower somatic cell count and is likely associated with reduction in prevalence of clinical mastitis among herds, which is economically beneficial [6].

The use of antimicrobials have, over time, increased the number of antimicrobial-resistant microbes globally, and any use of these agents will to some extent benefit the development of resistant strains and also inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute the most to the increase in antimicrobial resistance without improving the outcome of treatment [7].

In recent years, antimicrobial susceptibility testing has become under scrutiny because of concerns about antimicrobial resistance, changes in methodology and the relationship between *in vitro* results and on-farm clinical outcomes. Susceptibility tests of milk samples submitted to state diagnostic laboratories that use the disk-diffusion method have demonstrated remarkable agreement but vary from results of a small survey processed using broth dilution [8].

The aim of our study was the observed the occurrence of in herd of sheep with machine milking technology and determined the resistance of enterotoxigenic bacteria of *Staphylococcus* spp. isolated from milk samples during one milking season.

2 Materials and methods

Animals and milking

The experiment was carried out on herd of 450 sheep of Lacaune breed, which were housed in sheep stable from bricks with deep bedding during the winter period. Machine milking were performed in two-line milk parlour 2 x 14 Miele Melktechnik, (Hochreiter Landtechnik, Germany) two times per day during milking season (from April to September). The milk has been collected within the mobile tank of milk during milking and milk after milking was transported for further processing.

Examination of health status of sheep and milk samples

Complex examination of health status of udder in sheep was carried out at the start (April) and at the end of milking (September). The clinical examination was carried out according to Hariharan et al. [9] and milk from individual halves was evaluated by NK-test (Bioveta, Inovice na Hané, Czech Republic) according to Fthenakis [2].

Laboratory analyses

From the every individual milk sample were inoculated 0.05 ml, onto blood agar (Oxoid LTD, Hampshire, UK), and cultivated at 37°C for 24h. Based on the colony morphology, bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar, cultivated at 37°C for 24h and identified biochemical using the STAPHYtest, STREPTOtest, ENTEROtest (Erba-Lachema, Brno, Czech Republic) and identification by software TNW Pro 7.0 (Erba-Lachema, Brno, Czech Republic).

Antimicrobial susceptibility test

All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to identify the most effective drugs for mastitis treatment in the study

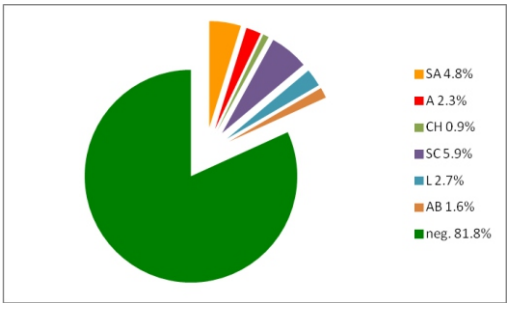
area [7]. The sensitivity against Ampicillin (10 µg); Amoxycilin (25 µg); Cloxacilin (5 µg); Cefaperazone (30 µg); Erytromycin (10 µg); Linkomycin (15 µg); Neomycin (10 µg); Novobiocine (5 µg); Penicillin (10 U); Streptomycin (10 µg); Methicilin (10 µg); Oxacilin (5 µg); Cephalotin (30 µg) (Oxoid Ltd. Basingstoke, Hants, UK) were determined on Mueller Hinton agar (HiMedia, Mumbai, India) as described by National Committee for Clinical Laboratory Standards [10]. The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant. Statistical evaluation of data was done by Excel program.

3 Results

From total number of sheep (n = 883) in Graph 1 and Table No. 1, on the base of complete examination were determined 18.2 % occurrence of mastitis (n = 161) during one milking season. The most frequently were found subclinical (5.9 %), subacute (4.8 %) and latent (2.7 %) forms of mastitis in 118 sheep (13.4 %).

Acute forms formed 2.3 % (23 infected sheep). The highest ratio had CNS, which occurrence was determined in 86 cases (9.7 %), at which caused frequently subclinical (4.4 %) subacute (3.2 %) and latent (1.5 %) mastitis. From CNS, *S. chromogenes* (2.4 %), *S. schleiferi* (2.1.9 %) and *S. caprae* (1.1 %) were frequently isolated from positive samples of milk. Bacteria *S. aureus* (1.5 %) and *S. uberis* (1.3 %) were isolated from acute and subacute forms of mastitis.

Medium value of antibiotic resistance of *Staphylococcus* spp. (n = 103) isolated from sheep's milk were determined to Penicillin (11.7 %), Cloxacilin (11.7 %), Ampicillin (10.7 %) and Oxacilin (10.7 %). Low value of resistance we found to 8 tested antibiotics. Lower antibiotic resistance was determined only to Methicilin (0.9 %). In *S. chromogenes* and *S. aureus* were founded three strains, which showed common resistance to Penicillin and Oxacilin (Table 2 and 3).



Legend: SA – subacute mastitis, A – acute mastitis, CH – chronic mastitis, SC – subclinical mastitis, L – latent mastitis, AB – abacterial mastitis, neg. – negative

Graph 1: Occurrence of mastitis in herd of sheep during milking season

Pathogens	∑	%	SA	A	CH	SC	L
S. chromog.	21	2.4	0.7	0.3	0.2	0.9	0.3
S. schleiferi	18	2.1	0.5	0.0	0.0	1.2	0.3
S. aureus	17	1.9	0.5	1.0	0.1	0.1	0.2
S. epider.	15	1.7	0.8	0.1	0.0	0.6	0.2
S. uberis	14	1.6	0.6	0.7	0.1	0.2	0.0
E. coli	11	1.2	0.2	0.1	0.0	0.5	0.5
S. caprae	10	1.1	0.7	0.0	0.0	0.2	0.2
E. faecalis	9	1.0	0.2	0.0	0.0	0.5	0.3
S. simulans	8	0.9	0.1	0.0	0.0	0.6	0.2
S. gallinar.	8	0.9	0.5	0.0	0.0	0.3	0.1
S. felis	6	0.7	0.0	0.1	0.1	0.6	0.0
Others	10	1.1	0.2	0.1	0.3	0.2	0.2
positive	147	16.6	**4.8**	**2.3**	**0.9**	**5.9**	**2.7**
negative	736	83.4					
Total	883	100.0					

S. chromog. – S. chromogenes, S. gallinar. – S. gallinarum, SA – subacute mastitis, A – acute mastitis, CH – chronical mastitis, SC – subclinical mastitis, L – latent mastitis

Table 1: Bacterial agents of ovine mastitis

Medium value of antibiotic resistance of Staphylococcus spp. (n = 103) isolated from sheep's milk were determined to Penicillin (11.7 %), Cloxacilin (11.7 %), Ampicillin (10.7 %) and Oxacilin (10.7 %). Low value of resistance we found to 8 tested antibiotics. Lower antibiotic resistance was determined only to Methicilin (0.9 %). In S. chromogenes and S. aureus were founded three strains, which showed common resistance to Penicillin and Oxacilin.

Staphylococcus spp.	n	AP	AM	CL	OX	ER	LN	NE
S. chromogenes	21	2	2	3	3	2	2	1
S. schleiferi	18	2	1	2	2			2
S. aureus	17	3	3	2	3	2	2	2
S. epidermidis	15	1	2	1	2		1	1
S. caprae	10	1	1	1		1		1
S. simulans	8	1		1		1		
S. gallinarum	8		1	1	1			
S. felis	6	1		1				1
Total	**103**	11	10	12	11	6	5	8
Value of resistance %		10	9.4	11.7	10.7	5.9	4.9	7.8
		M	**L**	**M**	**M**	**L**		**L**

AP - Ampicillin (10 µg); AM - Amoxycilin (25 µg); CL - Cloxacilin (5 µg); OX - Oxacilin (5 µg); ER - Erytromycin (10 µg); LN - Linkomycin (15 µg); NE - Neomycin (10 µg); V – very low; L – low resistance; M – medium resistance

Table 2: Resistance to antibiotics in *Staphylococcus* spp. (n=103) isolated from milk samples of sheep

Staphylococcus spp.	n	PE	ST	MT	CP	CE	NO
S. chromogenes	21	3	2		2		1
S. schleiferi	18	2	2		1		1
S. aureus	17	3	2	1	2	1	2
S. epidermidis	15	2	1		1		
S. caprae	10	1					
S. simulans	8	1	1				
S. gallinarum	8				1	1	
S. felis	6						
Total	103	12	8	1	8	3	4
Value of resistance %		11.7	7.8	0.9	7.8	1.9	3.9
		M	**L**	**V**	**L**	**L**	**L**

NO - Novobiocine (5 µg); PE - Penicillin (10 U); ST - Streptomycin (10 µg); MT - Methicilin (10 µg); CP - Cephalotin (30 µg), CE - Cefaperazone (30 µg);V – very low; L – low resistance; M – medium resistance

Table 3: Resistance to antibiotics in *Staphylococcus* spp. (n=103) isolated from milk samples of sheep

4. Discussion and conclusion

Several authors in their studies from France and Spain recorded, that the species of *Staphylococcus* spp. belongs to general aetiological agents of intramammary infections in small ruminants (*S. aureus* in clinical cases and CNS in subclinical). From the CNS is more frequently *S. epidermidis* what is also determined in our study. No less important bacterial pathogens are *Corynebacterium* spp., *Enterococcus* spp. and *Micrococcus* spp. [11,12].

According to Ozenc et al [13], the general pathogens, which caused clinical mastitis in dairy sheep herds, are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus uberis*. Subclinical forms are refers to CNS, which often grows to subacute and acute forms. In compare with *S. aureus* have CNS lower frequency of virulence factors, however their representation in clinical forms of mastitis in sheep is becoming increasingly a problem in the holdings as confirmed in our study. From 147 isolated bacterial pathogens, 86 (9.7 %), were represented by CNS, which caused subclinacal (4.4 %), and subacute (3.2 %) forms of mastitis. Bacteria *S. aureus* (1.5 %) and *S. uberis* (1.3 %) were isolated predominantly from acute and subacute mastitis.

Subclinical mastitis is thought to have a prevalence of between 10 % and 30 % in Lowland flocks in Southern England [14]. From 450 sheep was determined 5.9 % incidence of subclinical mastitis. Most frequently isolated were CNS, predominantly *S. schleiferi* (1.2 %), *S. chromogenes* (0.9 %), *S. epidermidis*, *S. simulans* (0.6 %) and *S. felis* (0.6 %).

Hariharan et al. [15] showed 6.7 % positive samples of milk from 492 examinated sheep in which were isolated CNS. The most frequently isolated were *S. equorum, S. simulans* and *S. schleiferi* from ewes with subclinical mastitis during lactation.

In addition to high occurrence in cases of subclinical mastitis were in CNS determined medium antibiotic resistance to Penicillin (11.7 %) and to Cloxaciline (11.7 %).

Similar results of antibiotic resistance of *Staphylococcus* spp. from milk samples recorded Hisira et al. [16] Bacteria *S. chromogenes*, *S. epidermidis* and *S. xylosus* were characterized by resistance to Penicillin, Novobiocine and Neomycin. Low value of resistance was determined to Methicillin and Cefoxitin.

In our study three strains *S. aureus* and *S. chromogenes* demonstrated resistance to Penicillin and Oxacilin. Results were consistent with a previous report from Poland, where strain of *S. aureus* and *S. epidermidis* were resistant to Ampicillin and Penicillin [17]).

By complex examination in herd of sheep we determined 7.1 % occurrence of subacute and acute forms of mastitis caused predominantly by CNS, *S. aureus* and *S. uberis*. Very important is the early diagnosis of mastitis in sheep during the milking season. At the start of the treatment of subclinical mastitis showing the subclinical forms of mastitis can significantly eliminate clinical stage of subacute and acute forms of mastitis.

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