RESEARCH ARTICLE

The Occurrence of Bacterial Agents Causing Mastitis in Dairy Sheep and Their Resistance to Antibiotics

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Abstract:
Introduction: The aim of this study was to monitor the occurrence of bacterial agents causing mastitis, forms of mastitis and antibiotic resistance in 300 ewes on a farm in Eastern Slovakia.

Material and methods: During the milking season, were performed three complex investigations including clinical examination, California Mastitis Test and laboratory analysis of milk samples. The investigations and milk samples were taken in three phases; the beginning (April), the middle (June) and the end (September) of the milking season.

Results: Of all the samples (806), 225 (28.0%) were positive for bacterial pathogens. The highest incidence of mastitis (33.3%) was recorded in September, while April (23.8%) and June (25.3%) had lower incidence. The samples from September showed the highest incidence of subclinical mastitis (20.1%), with 13.2% being an acute form of mastitis. Coagulase-negative staphylococci (CNS) were identified in 61.7% of the positive samples. Especially, S. chromogenes, S. epidermidis and S. schleiferi were most frequently isolated. Staphylococcus aureus was the causative agent in 20.0% of the positive samples and caused acute or subclinical mastitis in the affected ewes. The tested bacteria showed very high resistance to Novobiocin (59.5%) and Penicillin (51.4%) and high resistance to Amoxicillin (35.1%). We found that 80% of Staphylococcus aureus bacteria tested for antibiotic resistance were resistant to Novobiocin and 70% were resistant to Penicillin. Of all tested CNS, 56.5% were resistant to Novobiocin, 39.1% to Penicillin, and 34.7% to Amoxicillin.

Conclusion: Proper isolation and identification of the causative organism play a significant role in the prevention and control of the intramammary infection. In our study, a combinations of Streptomycin, Ciprofloxacin and Tetracycline were the most effective antibiotics for the control of mastitis.

Keywords: sheep, mastitis, resistance, S. aureus, coagulase-negative staphylococci
THE OCCURRENCE OF BACTERIAL AGENTS CAUSING MASTITIS IN DAIRY SHEEP AND THEIR RESISTANCE TO ANTIBIOTICS

1 | INTRODUCTION

Mastitis can be a huge problem for the farmer and animals in the milk industry. The reduced milk quality and quantity causes production losses and acute clinical mastitis is a painful condition and therefore an important animal welfare issue (1), (2). Great production losses are also due to subclinical forms of mastitis, that needs laboratory test to be confirmed. The disease is normally a consequence of a bacterial infection. Mastitis affects the composition of the milk and the degree of changes to the udder depends on the infecting agent and the inflammatory response. Indicators of inflammation in the milk can be determined using the California mastitis test at the farm or by somatic cell count in a laboratory. Culture of milk sample can be done to find the specific bacterial pathogens causing the mastitis infection (3).

The prevalence of mastitis in ewes range from 4.0 to 50.0%, with clinical intramammary infections generally being less than 5% (4). Staphylococci are the main causative agents of mastitis in sheep and goats. Staphylococcus aureus is thought to be the main cause in acute infections and coagulase-negative staphylococci (CNS) are thought to be the main cause in subclinical infections (4), (5).

The treatment of affected udders with antibiotics will give a valuable increase in milk quantity and quality and decrease in somatic cell counts that is thought to be associated with the reduction in clinical mastitis on the farm (6). But treatment and control of mastitis is challenging, due to the emerging antibiotic resistant bacteria and subclinical forms of the disease (7). Many bacteria have recently become resistant to β-lactamase antibiotics, macrolides and lincosamin that contributed to the development and use of methicillin and oxacillin as anti-staphylococcal penicillin. Because of the increasing resistant of bacteria to antibiotics, the endangered efficacy of antibiotics will probably make treatment of mastitis even harder in the future (8).

2 | METHODS

2.1. Animals and milking

The localisation of the farm with 300 dairy sheep is in the east of Slovakia. The breeding consists of improved Wallachian sheep, Lacaune sheep and their crosses. The farm consists of two stables, a hayloft, areas for the staff, the milking parlour and a collection container for milk. The milking period starts in the middle of March after lambing. Initially the sheep milk once a day (in the afternoon) when they still have their lambs. One month later, in the middle of April the sheep are released onto pasture and the milking starts twice a day. The sheep will milk twice a day until end of September, making the milking season approximately six months long.

Before the milking starts the sheep are gathered outside the sheepfold and are herded into the milking parlour Boumatic 2x12 in groups (Fig. 1). The milking process lasts on average two minutes for one sheep with a maximum of four minutes. The pressure in the milking machine is 40.5 kPa and the pulsation rates at 120-140 cycles of liner opening and closing per minute. In 1-2 minutes after milking a disinfectant Filmodip (Agronom, SK) with active substance acid lactic is used as teat dip.

![Sheep milking](https://doi.org/10.15520/jmrhs.v3i7.215)

**FIGURE 1:** Sheep milking

Source: Zigo F (2018)

2.2. Udder health examination and milk sampling

Practical part of study and sampling was performed during three complex investigations at the beginning (April), middle (June) and end (September) of the milking season.

**Supplementary information** The online version of this article (https://doi.org/10.15520/jmrhs.v3i7.215) contains supplementary material, which is available to authorized users.
A thorough evaluation of udder health included clinical examination, sensory analysis of milk from forestripping of each udder half followed by the assessment of CMT (Indirect Diagnostic Test, Krause, Denmark). Milk from every quarter was mixed with the reagent and the result was scored as negative, trace, or positive (score 1 – 3) depending on the formation of gel in the milk sample according to Fthenakis (9) . Next, were aseptically collected a milk samples for bacteriological cultiva-tion in accordance with the guidelines of the National Mastitis Council (10) . The samples were cooled to 4 ◦C and immediately transported to the laboratory and analyzed on the following day.

2.3. Microbiological examination and testing of the sensitivity on antibiotics

Milk samples (10 µL) were cultured at the respective veterinary practice according to routine procedures, usually employing Columbia Blood Agar Base with 5% of defibrinated blood, Staphylococcal medium N° 110, Baird-Parker agar, Edwards Medium, Mac Conkey Agar (Oxoid, OXOID Ltd., Basingstoke, Hants, UK), and incubation at 37 ◦C for 24 h. As well as evaluating bacterial growth characteristics other assays were used to identify bacterial species: pigment and coagulase production, catalase activity, haemolysis, Gram staining and other virulence factors. Staphylococcus spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspected colonies of Staphylococcus spp., Streptooccus spp. and Enterobacteriaceae spp. were isolated on blood agar and cultivated at 37 ◦C for 24 h and identified biochemically using the Staphy test, Strepto test and resp. Enterobacteriaceae were identified using a software made by Erba-Lachema, CZ with a probability of correct designations of the kind above 90%.

Isolated bacteria from milk samples were tested in vitro by a disc method EUCAST (11), by evaluation of the zones of inhibition to grow on Mueller-Hinton agar after 24 hours incubation at 37 ◦C. To the test of sensitivity of staphylococci to 9 antibiotics have been use test discs (OXOID Ltd., Basingstoke, Hants, UK). The choice of antibiotics reflects the range of which is contained in a number of intramammary products to treat mastitis, which are available in Slovakia. The antibiotics used in this study were Ampicillin (AMP 10), Amoxicillin (AML 25), Ce-foxitin (FOX 30), Neomycin (N 10), Novobiocin (NV 5), Penicillin (P 10 U), Streptomycin (S 10), Ciprofloxacin (CIP 10) and Tetracycline (10).

Sensitivity or resistance of the bacteria tested were interpreted according to the reference zones inaccordance with the instructions of the EUCAST (11). The value of resistance was valuated as negligible (0.1%), very low (0.1-1%), low (1-10%) medium (10-20%), high (20-50%), very high (50-70%) and extremely high (>70%).

2.4. Statistical analyses

The differences in the prevalence of mastitis and distribution of bacterial pathogens were statistically analyzed using the Chi-square test. The dependence of the individual signs was tested at a significance level α = 0.05, with critical value = 5.991.

3 | RESULTS AND DISCUSSION

The incidence of mastitis in sheep farms are extremely variable. Fthenakis (9) found the occurrence of mastitis in sheep to be between 4-50%. In our study, the incidence of mastitis at the beginning of the season was 23.8%, in the middle 25.3% and at the end 33.3%. In all three cases, the most frequently diagnosed was the subclinical form of mastitis. There was a significant increase in the number of positive ewes as well as clinical mastitis at the end of the milking season (Graph 1).

![FIGURE 2: Occurrence of mastitis during three examinations](image-url)

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Note: Subclinical mastitis - no signs are observed, the udder and milk appears normal, but infection is still present with positive CMT score and increased SCC; Clinical mastitis – signs range from mild to severe with positive CMT score, high level of SCC, positive bacteriological cultivation, changing the consistency of the milk with the presence of flakes, clots or pus and reduction or loss of milk production with clinical signs, *Significant difference p <0.05 when significance level α = 0.05 (5%); critical value χ²=5.99.

Study from British slaughterhouses reported very high prevalence of clinical mastitis ranging from 13- 50%. This suggest that mastitis is an major cause of culling of ewes in the UK (12) . In our study 225 (28.2%) samples out of 806 were positive for bacterial pathogens causing mastitis. Staphylococci were most often isolated from positive milk samples during all three monitoring periods. CNS and S. aureus was identified in 61.7% and 20% of all the positive samples, respectively. In addition, Streptococcus sanguinis (11.2%) and Enterococcus faecalis (7.1%) being the major bacterial cause from positive milk samples (Table 1).

Coagulase-negative staphylococci are major pathogens in the dairy sheep industry and have frequently been reported as the most commonly isolated pathogens from cases of subclinical mastitis (13) . Subclinal forms of mastitis are referred to CNS, which often grows to subacute and acute forms. CNS have lower frequency of virulence factors in comparison with S.aureus, but they are beginning to be a growing problem with the occurrence of clinical mastitis (5) .

Thorberg et al. (14) isolated CNS (S. epidermidis and S. chromogenes) from raw milk of dairy ruminants. The same bacteria were also isolated from skin of the people that milked the dairy animals because isolation of S. epidermidis from human skin is more common than isolation from skin of udder. The authors conclude that humans are probably the main source of infection with coagulase-negative species during milking.

**TABLE 1:** Bacterial pathogens causing mastitis from all threesamplings

<table>
<thead>
<tr>
<th>Bacterial pathogens</th>
<th>April</th>
<th>June</th>
<th>September</th>
<th>All three samplings</th>
<th>All three samplings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>3</td>
<td>1.4</td>
<td>26</td>
<td>8.7</td>
<td>30</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>6</td>
<td>2.9</td>
<td>11</td>
<td>3.7</td>
<td>15</td>
</tr>
<tr>
<td>S. aureus</td>
<td>26</td>
<td>12.4</td>
<td>13</td>
<td>4.3</td>
<td>6</td>
</tr>
<tr>
<td>S. schlesierti</td>
<td>7</td>
<td>3.3</td>
<td>20</td>
<td>6.7</td>
<td>4</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1.0</td>
<td>19</td>
</tr>
<tr>
<td>Strept. sanguinis</td>
<td>2</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ent. faecalis</td>
<td>6</td>
<td>2.9</td>
<td>3</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>50</td>
<td>23.9</td>
<td>76</td>
<td>25.3</td>
<td>90</td>
</tr>
</tbody>
</table>

Note: * p <0.05 – significant difference.

Increasing the consumption of antibiotics in veterinary and human medicine is in the last period, accompanied by the phenomenon of an increase in bacterial resistance. Mastitis resistance is a complex and multifactorial trait, and its expression depends on both genetic and environmental factors including infection pressure. Resistance to antibiotics may be acquire by spontaneously occurring genetic muta-tions and more commonly by the horizontal transfer of mobile DNA elements from a donor cell to another bacterial species (15) .

From all tested positive milk samples, staphylococci were observed very high resistance to Novobiocin (59.5%) and Penicillin (51.4%) with highest number of bacterial isolates, followed by high resisency to Amoxycillin (35.1%) (Table 2). In a study performed by Kunz et al. (16) showed that of the 67 and 208 of S. aureus and CNS strains, 31.3% and 8.2% were resistant to Penicillin, 29.9% and 1.0 % to Ampicillin, 1.5% and 10.6% to Erythromycin and 3.0% and 7.7% to Tetracycline, respectively.
4 | CONCLUSION

Antibiotic resistance is one of the important problems encountered in the treatment and control of mastitis. Antibiotic susceptibility tests should be done to determine the effectiveness of drug that can be used for successful treatment of diseases. Proper isolation and identification of the causative organism play significant role in prevention and control of the intramammary infection. In our study a combinations of Streptomycin, Ciprofloxacin and Tetracycline were the most effective antibiotics for control of mastitis.

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