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RESEARCH ARTICLE

Mastitis pathogens isolated from samples of milk in dairy cows herds of Slovak spotted cattle

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Abstract

Breeding of ruminants with market milk production represents a significant proportion of livestock production in Slovakia. Mastitis is one of the biggest problems of dairy producer's cause's great losses in the livestock economy. Cows that are infected with mastitis generally produce less milk, use longer time to get pregnant, lose more body condition, and are also more likely to be culled early. Besides affecting production and the profitability it also has a major impact on the welfare of the cows.

The aim of this study was to evaluate occurrence and etiology of mastitis in two dairy herds of Slovak spotted cattle. The diagnosis of mastitis was performed based on 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 904 and 612 quarter cow's milk samples were 26,1% and 13,5% positive to CMT, respectively. The prevalence of intramammary infection (IMI) in the monitored herds of cows was 17.0% to 12.4%, respectively. In both herds were confirmed predominantly subclinical forms of IMI. The highest percentage of etiological agents in all monitored herds had coagulase negative staphylococci (CNS) and coagulase positive staphylococci (CPS) especially *Staphylococcus aureus*. Except for staphylococci were *E. coli*, *Aerococcus viridans* and *Streptococcus* spp. most frequently pathogens isolated from dairy cows. Given the variety of factors causing IMI milk production and economic prosperity of dairy herds will primarily depend on the expertise and skills to implement preventive anti-mastitis methods, and technological systems to own agricultural production.

Key words: Slovak Spotted Cattle, Cows, Milking, Mastitis, Bacterial Pathogens, *Staphylococcus* Spp.

Abbreviations: **CMT:** Californian Mastitis Test; **IMI:** Intramammary Infection; **CNS:** Coagulase Negative Staphylococci; **CPS:** Coagulase Positive Staphylococci; **SCC:** Somatic Cell Count.

Introduction

In livestock production has a very important role in cattle breeding, especially for the production of milk and meat, which have an essential function in human nutrition. In the Slovakia, the greatest interest in the economy is in breeds of cattle with market milk production [1].

Such breeds include Holstein cattle and Slovak spotted cattle. Compared to Holstein cattle, Slovak spotted cattle is characterized by a combined utility type and longevity with good quality milk parameters. This breed was created by crossing red cattle with mixture of steppe and brown Carpathian cattle with Bernese and Simental cattle. As a national breed was recognized 50 years ago and registered in Slovakia. The breed has a large body frame, solid body structure, strong constitution, good health and high durability. Cows weigh 600-800 kg and bulls up to 1200 kg. The basic color for this breed is yellow spotted to red spotted. The head, abdomen, limbs, and tail tip are white but there are pigment places on the head (Figure 1). Milk yield in this breed is quite good, in farms reach more than 7000 kg of milk per lactation [2].

The economic value of dairy cows is determined mainly by their milk yield and longevity, because milk is the main source of income on Slovak dairy farms. One of the important factors affecting the quantity and quality of milk produced is the occurrence of production diseases, especially mastitis [3].

Mastitis is the inflammation of the mammary gland and udder

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Figure 1: Herd of Slovak spotted cattle

tissue. It is usually an immune response after a bacterial invasion in the teat canal by a wide variety of bacterial sources present on the farm or as a result of chemical, mechanical, or thermal injury to the udder of the cow. With the inflammation follows an increase in the level of white blood cells or leukocytes, and this causes an increase in the somatic cell count (SCC) of the milk. The leukocytes are produced as a response to the injury or infection, and they are a crucial part in repairing tissue damage and eliminating infectious agents [4].

IMI is known as a multifactorial disease, and it is closely related to the production system and the environment that the cows are kept in. The mastitis risk factors are divided into three groups: host, pathogen and environmental determinants. There are three types of mastitis: latent, subclinical (SM) and clinical (CM). Latent mastitis are characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency and SCC. Subclinical mastitis are characteristic with positive CMT score, bacteriological cultivation, increased SCC, reduced milk yield without clinical signs. During SM the udder and milk appears normal, but infection is still present. Due to the lack of symptoms, SCC can be used to indicate the prevalence of mastitis. In CM the clinical signs are clear. The most prominent symptoms of CM are swelling, heat, hardness, redness or pain of the udder. The milk of a cow with CM has a watery appearance, and flakes, clots or pus is often present. Other signs include reduction of milk yield, increased body temperature, loss in appetite, and a reduction in mobility due to the pain from the swollenness of the udder [4, 5].

Bacteria are the major source of mastitis. They enter the udder through the teats, and when the infection once has established in one cow it can quickly be spread to other cows through the milking equipment. There are two groups of bacteria that cause mastitis. The first one is responsible for most of the outbreaks. This type causes both clinical and subclinical mastitis. The second group is only responsible for a small proportion of the occurrences, and it only causes subclinical infections. From the first of most common causative agents of mastitis are *Staphylococcus aureus* and *Streptococcus agalactiae.* Their reservoir is the cow itself, and as these bacteria is contagious, meaning they can be spread from cow to cow. The infection is often chronic, as SM keeps the infection within the herd. The second group of environmental pathogens may also cause mastitis. This particularly includes the coliforms *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp. Some pathogens such as coagulase-negative staphylococci and *Corynebacterium bovis* can also cause moderate inflammation and SCCs of about two to three times that of regular milk. The major pathogens causing mastitis infection elevates the SCC significantly to many times above that of regular milk. The SCC of normal milk will be below 200 000 cells, often even below 100 000 cells [6, 7].

The polyetiological and multifactorial origin of mastitis in dairy cows means that the effectiveness of generally applicable anti-mastitis methods in the reduction of environmental mastitis bacteria, in combination with the main contagious pathogens of the mammary gland tends to be limited. Therefore, the aim of this study was to evaluate the occurrence and etiology of mastitis in two dairy cow herds of Slovak spotted cattle.

Materials and Methods

Herds of dairy cows

The practical part of study was realized in two dairy farms situated in east and west of Slovakia with standard zootechnic and zoohygienic conditions. Herds size ranged from 270 to 180 dairy cows of Slovak spotted cattle during 1st - 4th lactation were used. Dairy cows from both farms were kept in a free housing system with a separate calving barn and they were equipped with individual boxes with bedding and were allowed ad libitum access to water. All cows were fed total mixed ration complete mixture based on grass silage, maize silage and concentrate. Their diet was formulated according to international standards NRC [8] to meet the nutritional requirements of a 650 kg cow, yielding 15 - 20 kg of milk/d.

Milking cows and milking procedures

On the first farm is located fishing milking parlour (2x10). The dairy cows were milked twice a day, with the first milking starting at 4.30 am in the morning and the second afternoon at 4.30 pm. First, the wet toilet was performed with water to remove impurities from udder and teats. Subsequently,

the udder was thoroughly wiped disposable wipes soaked in disinfectant solution. The first milk from each quarter were hand-drawn into a dark-bottomed pot, and the milk was sensitively assessed. Milking a single cow using a milking device took 4-7 minutes and termination was automatically signaled when the milk flow dropped to 0.2 l/min. After milking process, the teats were disinfected in the form of teat-dipping with Ioderm 5000 (Hypered Czech s.r.o.). Milk was stored in refrigerating milk tanks at + 5 °C and removed daily around 11.30 hrs.

The cows from second herd were milked twice a day; at 5.00 am and 4.00 pm in tandem milking parlour DeLaval 2x5 (Tumba, Sweden). After loading of the cows, the udder and teats were cleaned and disinfected. The cleaning was done using a clean cloth dipped in Dermisan Plus, which is composed of diaminopropyllaurylamine (Agromont, Nitra, SR), and cleaned for at least 10-20 seconds. After cleaning, the first sample of milk was evaluated, and if no changes were observed, the milking process could start. The duration of milking was 4-8 minutes, and milking was automatically stopped when the flow of milk was lower than 0, 2 l/min, due to decreased pressure. One minute after completed milking, the teats were disinfected with a 2nd disinfection Ino Star, which contains iodine (Agromont, Nitra, SR). At the end of the process, the milk was stored in a tank with temperatures between 4-6 ºC.

Examination of health status and samples collection

The examination of health status included clinical examination of the mammary gland, examination fore-strip of milk, with CMT reaction, subsequent collecting of milk samples for bacteriological examination with the cultivation and identification of pathogenic bacteria. CMT (Indirect diagnostic test, Krause, Denmark) was performed on all quarter milk samples from the milked cows and the score was evaluated (Figure 2) according to Jackson and Cockcroft [9].

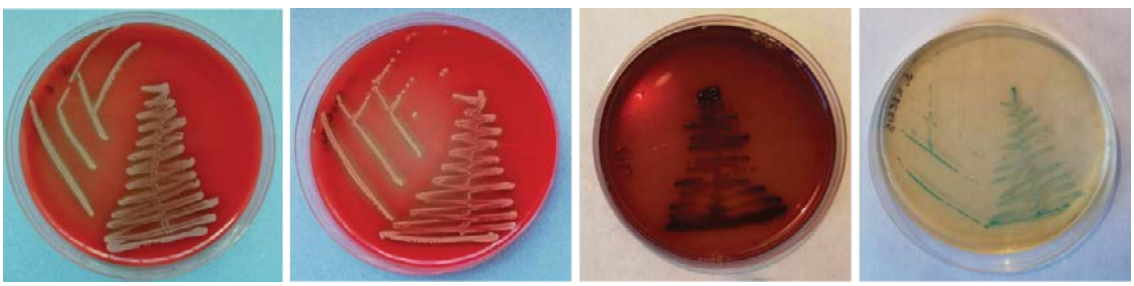
Quarter cow's milk samples of the secretion (10 ml) were then collected with aseptic techniques in accordance with National Mastitis Council guidelines [10]. The samples were cooled and immediately transported to the laboratory of University of Veterinary Medicine and Pharmacy in Kosice.

Laboratory analyses

The milk samples were investigated for bacterial pathogens according to commonly accepted rules Malinawski [11] and Malinowski et al. [12]. Milk samples were (10 μl) incubated at 37 °C for 24 hrs by primo-cultivation on blood agar base with 5% of defibrinated blood and all growing isolates were characterized by classic microbiological methods and consistent cultivation on specific cultivation media: Staphylococcal medium N° 110, Baird-Parker agar, Edwards Medium, Mac Conkey Agar (Oxoid, Ltd., Basingstoke, Hants, UK) (Figure 3).

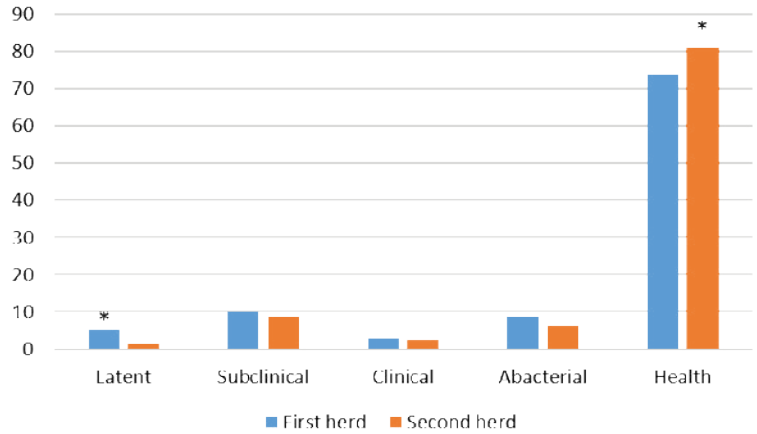
In addition to evaluation of bacterial growth characteristics another assays were used to bacterial species determination: pigment and coagulase production, catalase activity, hemolysis, Gram staining and other virulence factors. Bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar and cultivated at 37 °C for 24 h and detailed identified biochemically using the STAPHY-

Figure 2: Milking and evaluation of CMT



From left: S. aureus, S. warneri, Str. bovis, Str. uberis cultured on selectives medium

Figure 3: Bacteriology analysis



Graph 1: Comparison of mastitis forms in monitored herds (%)

Note: Latent mastitis-are characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency and SCC, Subclinical mastitis-are characteristic with positive CMT score, bacteriological cultivation, increased SCC, reduced milk yield without clinical signs. Clinical mastitis-are characteristic with positive CMT score, bacteriological cultivation, high level of SCC, changing the consistency of the milk, reduced or loss of milk production with clinical signs. Abacterial mastitis-are characteristic with positive CMT score without bacteriological cultivation, *-values above the column differ significantly at $p < 0.05$

test, STREPTO-test, resp. ENTERO-test and identification by software TNW Pro 7.0 (Erba-Lachema, CZ) with precision of detection over 90.0%.

Statistical analysis

The differences in the prevalence of mastitis among herds of cows were statistically analyzed using the Chi-square test. The level of significance was set to $P < 0.05$.

Results and Discussion

The evaluation of CMT with collection of milk sample for the detection of bacteria and SCC, are routinely use in the laboratory diagnosis. From total 904 and 612 quarter cow's milk samples were 26.1% and 13.5% positive to CMT, respectively. The prevalence of intramammary infection (IMI) in the monitored herds of cows was 17% to 12.4%, respectively (Table 1). Currently, the biggest problem farmers in dairy herds of Slovakia when it comes to mastitis are the fact that in most of the cases with IMI, the results were subclinical mastitis. As shown in table 2, in first herd, 92 out of 156 of the infected quarters were cases of SM. In second herd, as shown in table 3, 53 out of 76 infected quarters were SM.

At the present time, the most prevalent microorganisms isolated from herds in dairy cows of Slovak spotted cattle are the CNS, *Staphylococcus aureus*, coliforms, and *Streptococcus* spp. In monitored dairy cow herds from 156 and 76 infected quarter cow's milk samples were isolated CNS, followed by *S. aureus*, streptococci, and *E. coli* and *Aerococcus viridans*, respectively (Table 2 and 3).

In Slovakia the problem of environmental mastitis has gradually increased since 2000. First, there were mainly inflammations of the udder caused by *Streptococcus uberis, Escherichia coli* and bacteria from the family *Enterobacteriaceae*. Gradually the cases induced by CNS and *Pseudomonas* spp., but also bacteria *Proteus* spp., and the incidence of mastitis induced by *Arcanobacterium pyogenes* and *Streptococcus dysgalactiae* have increased [13].

In our study, CNS accounted for 43.4% and 50.0% in herd A and B, respectively, of the isolated bacteria in infected

Table 1: Examination of herds in dairy cows

Monitored herds	Herd 1		Herd 2		Total
	n	%	n	%	n
Healthy quarters	668	73.9	497	82.2	1165
Positive quarters1	232	26.1a	83	13.5b	315
Infected quarters2	154	17.0a	76	12.4b	230
Rejected quarters	4	0,4	6	0.9	10
All examined quarters	904	100	612	100	1510
Total dairy cows in herd	**226**		**153**		**379**

Note: Positive quarters1 - evaluation of CMT test with score 1-3; Infected quarters2 - evaluation on the basis of positive microbiological cultivation of milk samples. a, b – values in row with different superscript letters differ significantly at $P< 0.05$.

Table 2: Isolated microorganisms from infected quarters in first herd

Isolated microorganisms	n	%	Latent		Subclinical		Clinical	
			n	%	n	%	n	%
Staphylococcus spp.								
S. aureus	21	13.5	5	3.3	7	4.5	9	5.8
S. chromogenes	21	13.5	3	1.9	14	9.1	4	2.6
S. epidermidis	16	10.3	7	4.5	9	5.8		
S. warneri	12	7.8	2	1.3	8	5.2	2	1.3
S. intermedius	10	6.5	3	1.9	7	4.5		
S. sciuri	10	6.5	5	3.3	5	3.3		
S. piscifermentans	8	5.2			8	5.2		
Streptococcus spp.								
Str. sanguinis	4	2.6			1	0.7	3	1.9
Str. bovis	7	4.5			5	3.3	2	1.3
Str. uberis	3	1.9					3	1.9
Other bacteria								
E. coli	9	5.8	4	2.6	5	3.3		
Aerococcus viridans	9	5.8	2	1.3	7	4.5		
Bacillus spp.	8	5.2	1	0.7	7	4.5		
Enterococcus faecalis	6	3.9	4	2.6	2	1.3		
Mixed infection*	12	7.8	1	0.7	7	4.5	4	2.6
Total	**156**	**100**	**37**	**23.7**	**92**	**59**	**27**	**17.3**

Note: n-number of positive samples isolated from dairy cows; Mixed infection*- mixed infection caused by two or more bacteria.

Table 3: Isolated microorganisms from infected quarters in second herd

Isolated microorganisms	n	%	Latent		Subclinical		Clinical	
			n	%	n	%	n	%
Staphylococcus spp.								
S. aureus	9	11.8			4	5.3	5	6.6
S. chromogenes	14	18.4	5	6.6	8	10.5	1	1.3
S. haemolyticus	10	13.2			8	10.5	2	2.6
S. warneri	8	10.5	1	1.3	7	9.2		
S. xylosus	6	7.9			6	7.9		
S. intermedius	6	7.9	2	2.6	4	5.3		
Streptococcus spp.								
Str. sanguinis	7	9.2			3	3.9	4	5.3
Str. faecalis	2	2.6			2	2.6		
Other bacteria								
E. coli	4	5.3	1	1.3	2	2.6	1	1.3
Aerococcus viridans	3	3.9			3	3.9		
Enterobacter aerogenes	2	2.6			2	2.6		
Mixed infection*	5	6.6			4	5.3	1	1.3
Total	**76**	**100**	**9**	**11.9**	**53**	**69.7**	**14**	**18.4**

Note: n-number of positive samples isolated from dairy cows; Mixed infection*-mixed infection caused by two or more bacteria.

quarters. This high result is supported by results from several other countries, including Finland [14] and Germany [15], where majority of the isolated bacteria belong to this group.

Pyörälä and Taponen [16] reported that a half of the bacteria isolated at environmental mastitis in dairy cows in Finland are the CNS and *S. aureus*. CNS and CPS were most common isolates from SM according to our study, which is generally seen as an increase in the SCC in milk of the infected quarter with positive CMT score. The economic losses are more associated with SM. However, the cost of treatment of SM is much low compared to that of CM which is accounting for 10 - 20 times higher.

Subclinical mastitis should be always suspected as one of the primary causes in cases of decreased milk production in dairy

herds. In fact, CNS, which are the most common etiological agents of SM, are also frequent inhabitants of the skin of the udder. Most mastitis occurs before the end of lactation (at the beginning of dry period) and also during the period surrounding parturition [16, 17].

In the case of CNS, there has been not only an increasing prevalence of such infections but also an expanding list of species reported to be involved in the process. Results of studies from different countries and continents revealed that more than 20 CNS species have been isolated from milk samples of mastitis cows, the most common being *S. chromogenes, S. haemolyticus, S. epidermidis, S. simulans S. warneri, S. sciuri* and *S. xylosus* [18, 19, 20]. In our study, the CNS isolated from the milk samples of the two herds include *S. chromogenes, S. epidermidis, S. warneri, S. sciuri, S. piscifermentans, S. haemolyticus*, and *S. xylosus*.

Staphylococcus aureus is an important pathogen, and accounted for 12.3% and 11.8% of the isolated bacteria from infected quarters in first herd and second herd, respectively. Several studies show the presence of *S. aureus* in infected quarters to be around 10-15 % of the bacteria isolated from milk samples [11, 14, 20, 21]. In some countries and studies, such as from Norway, Finland and England, the prevalence was even higher [22, 23, 24].

Conclusion

In monitored dairy cows herds of Slovak spotted cattle on the etiology of mastitis mainly participate bacteria of *Staphylococcus* spp. (mainly CNS and *S. aureus*), *Streptococcus* spp., *E. coli,* and *Aerococcus viridans*. From the point of view of the epidemiological importance, the highly contagious bacteria (*S. uberis* and *S. aureus*) that colonise udder, very quickly contaminate the hands of the milkers or milking machine, and are spread from the milking process exclusively. On the other hand, CNS represent a high risk for the formation of new, mainly subclinical infections of environmental origin.

Good routines for hygiene and treatment is the most important step for prevention of mastitis. This includes hygienic teat management, prompt identification and treatment, dry cow management and therapy, culling, regular testing and maintenance, and good record keeping. The hygienic teat management includes good housing management, effective teat preparation and disinfection to ensure good milk hygiene, teat health and disease control. The identification and treatment of clinical mastitis cases should be efficient and use the treatment most fitting and effective for the symptoms present. For dry cows the management and therapy should be so that the cows are dried of abruptly and the teats are cleaned meticulously before the dry cow antibiotic is administered. This includes the use of teat-end sealants if it is fitting. The culling of chronically affected cows should be performed if the cows have become impossible to cure, and thus represent a constant reservoir for infection for the other cows in the herd. The milking machines should be tested and maintained regularly with regular and recommended replacement of the teat cup liners, milking machine servicing, and attention paid to items which must be checked on a daily, weekly or monthly basis. Records should be kept of all aspects of the treatment of mastitis, the therapy of dry cows, service of milking machines, SCC and bactoscan results, and the cases of clinical mastitis.

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Conflict of interest

Declare if any financial interest or any conflict of interest exists.

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