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OCCURENCE OF MASTITIS IN DAIRY COWS SITUATED IN MARGINAL PARTS OF SLOVAKIA

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ABSTRACT

A relatively large part of the Slovak territory consists of the "marginal regions", which in terms of the economy of ruminants keeping can efficiently produce animal commodities only occasionally. The geographic, social and economic stability of these regions is strongly influenced by the rearing of ruminants and the associated market milk production. The aim of this study was to evaluate the prevalence and aetiological agents of mastitis in two herds of dairy cows situated in the marginal parts of Slovakia. In total, 530 of the Slovak spotted breed and Holstein cows were involved in the study. The diagnosis of mastitis was performed on the basis of the clinical examination of the udder, macroscopic evaluation of the milk, determination of somatic cell count (SCC), and the bacteriological examination of the milk. The prevalence of mastitis in the two herds of dairy cows ranged from 34.7 % to 18.8 %, respectively. From the total of 2120 quarter milk samples, 36.3 % were positive to the California mastitis test (CMT). Also, pathogenic microorganisms causing intramammary infection (IMI) were isolated from 25.6 % of the samples, which accounted for most subclinical mastitis forms (23.3 %), with the SCC under 400 000, mainly caused by coagulase-negative staphylococci (CoNS) and coliform bacteria *E. coli* and *Enterobacter aerogenes*. The clinical forms of mastitis accounted for 13.0 % of all infected cows and were caused mainly by the bacteria: *Streptococcus uberis*, *Streptococcus agalactiae*, *Staphylococcus aureus* and coagulase-negative staphylococci.

Key words: dairy cows; mastitis; milk production; prevalence; *Staphylococcus* spp.

INTRODUCTION

Marginal regions cover relatively a large part of the Slovakia territory where the rearing of ruminants can efficiently produce animal commodities only occasionally. The geographic, social and economic stability of these regions are strongly influenced by the keeping of ruminants, particularly for marking milk production. The products from

ruminants are unique, especially with regard to providing high quality nutrition to consumers. Many of the milk products and specialties can be included among the most important functional foods [16].

Mastitis is considered to be the most frequent and most costly production disease in dairy herds. Mastitis is characterized by an inflammation in one or more quarters of the udder and can be either clinical or subclinical. The decrease in milk production per cow due to the clinical and subclinical prevalence of mastitis is usually recognised as the main cause of economic losses due to the disease [4, 5, 6].

The factors influencing the susceptibility of the mammary gland to infections are: the presence of bacteria at the teat end, level of efficacy of the protective characteristics of the teat canal, and defence mechanisms in the udder (Fig. 1) [11].

Contagious mastitis is caused by organisms transmitted from cow to cow; the primary reservoir of which is the cow itself. The predominant contagious pathogens involved in bovine mastitis are *Staphylococcus* spp. (*S. aureus* and *S. epidermidis*) [9, 12], *Streptococcus* spp. (*Str. agalactiae, Str. dysgalactiae, Str. uberis* and *Str. bovis*), coliforms (mainly *E. coli* and *Klebsiella pneumoniae*), and *Enterobacter aerogenes* [14, 15].

Clinical cases give rise to visible symptoms such as swelling, heat, pain in the affected quarters or clots and discolouration in the secretions. Losses caused by clinical mastitis include: reduced milk yield and quality, costs of veterinary care, discarded milk and shortening of productive life [2, 5]. Cows with subclinical mastitis do not show a visible udder inflammation and can be diagnosed by somatic cell count (SCC) and by detection of the presence of the pathogens. The economic losses are more associated with sub-clinical mastitis which is 40 % more prevalent than clinical mastitis. However, the cost of treatment of sub-clinical mastitis is much lower compared to that of clinical mastitis, accounting for 10—20 times higher [5].

The aim of this study was to evaluate, during early lactation, the prevalence and aetiological agents in two herds of dairy cows situated in the marginal parts of Slovakia.

MATERIALS AND METHODS

Animals and milking

The study was conducted in accordance with good veterinary practice. The practical part of the study was carried out on two farms situated in marginal parts of Slovakia (Orava and Zemplín). On the first farm (A) we investigated 150 Slovak spotted breed cows and on the second (B) 380 dairy cows of the Holstein breed, between second and fourth lactations. The dairy cows on both farms were kept in a free housing system with individual boxes with bedding and ad libitum access to water and a separate calving barn. The keeping of cows corresponded to standard animal hygiene rules. Both herds were fed a total mixed rations based on grass silage, maize silage and concentrates.

The cows from both herds were milked twice daily, herd A in a tandem parlour DeLaval 2x5 (Tumba, Sweden) and herd B in a parallel parlour Boumatic 2x12 Xpressway (Wisconsin, USA). The average milk yield was 6800 l and 7400 l per year in herds A and B, respectively. Blanket dry cow therapy (treatment of all quarters of all cows) was implemented in both herds.

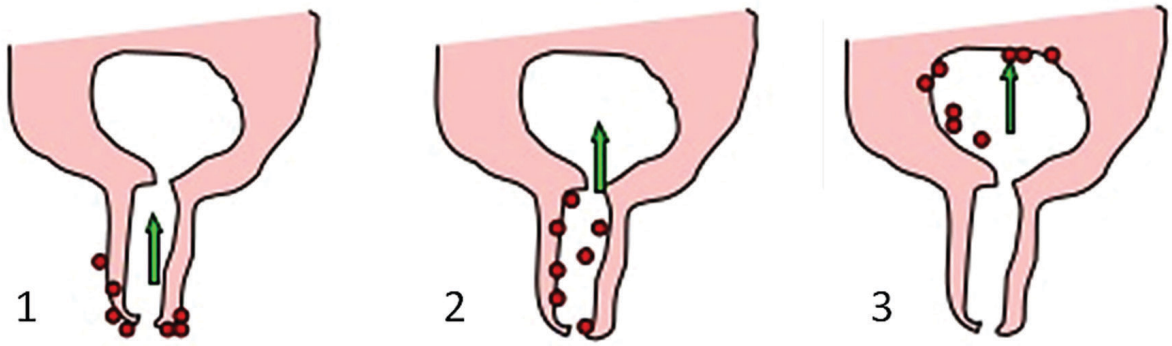

Fig. 1. Process of udder infection
1 — Organisms invade the udder through teat canal; 2 — Migrate up the teat canal and colonize the secretory cells; 3 — Colonized organisms produce toxic substances harmful to the milk producing cells. Source: Jackson and Cockcroft [3]

Collection of samples and laboratory analyses

Double milk samples were collected aseptically from 2120 quarters (530 cows) during the first month of lactation. Eleven quarters were atrophic. The teats were cleaned and the first few streams were discarded. The teats were then dipped in a disinfectant and the teat ends were wiped with alcohol swabs and allowed to dry. Then 10 ml of the milk was collected into sterile tubes. The samples were cooled and immediately transported to the laboratory. The SCC were analysed in a commercial laboratory using a MilkoScan FT2 (Foss Electric, Hillerod, Denmark).

The bacteriological examinations were performed according to commonly accepted rules [6]. Milk samples (0.05 ml) were inoculated onto blood agar (Oxoid, UK) and cultivated at 37 °C for 24 h. Based on the colony morphology and Gram staining, *Staphylococcus* spp. bacteria were selected for the tube coagulase test (Staphylo PK, ImunaPharm, Slovakia). Suspected colonies of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar, cultivated at 37 °C for 24 h and identified biochemically using the STAPHY-test, STREPTO-test, or ENTERO-test, using software TNW Pro 7.0 (Erba-Lachema, Czechia).

Individual forms of mastitis (subclinical, subacute and acute) based on clinical signs, CMT scores, SCC and bacteriological examination of milk samples were classified according to Vasiľ et al. [14].

Statistical analysis

The statistical analysis was performed using Graph-Pad PRISM 6.0 (GraphPad Software Inc., USA). The differences in incidence of mastitis among the herds were statistically analysed using the Chi-square test. The level of significance was set at $P < 0.05$.

RESULTS

The results obtained by the California mastitis test (CMT) are presented in Table 1. The elevation of SCC to the level below 200 000 ml^{-1} was detected in 63.2 % quarters on average. The average score (>1) of CMT above 400 000 ml^{-1} was detected in 13.1 % of the quarters. The prevalence of mastitis in the herds of dairy cows (A and B) was 34.7 % and 18.8 %, respectively.

The differences in the prevalence of subclinical and clinical forms of mastitis in the quarters between herds were statistically significant ($P < 0.05$). The prevalence of subclinical mastitis in the quarters of the two herds (A, B) reached 35.3 % and 20.6 % and of clinical mastitis 14.6 % and 6.8 %, respectively (Table 2).

The microorganisms isolated from mastitis in the herds are presented in Table 3. Pathogenic bacteria were isolated from 25.6 % of the quarters with subclinical (14.2 %), and (12.4 %) with clinical mastitis. The aetiological agents of mastitis detected most frequently were CoNS, *Str. agalactiae, Str. uberis, Enterococcus* spp. and *Bacillu*s spp. *Staphylococci* were the main aetiological agents of the intramammary infections (IMI) in the dairy herds and *Staph. Aureus, Str. uberis* and *Str. agalactiae* were the most frequent isolate from the clinical mastitis cases. In both herds, subclinical mastitis was caused mainly by *Enterococcus* spp., *Enterobacter aerogenes* and CoNS.

DISCUSSION

Bovine mastitis, as a major disease affecting dairy cattle worldwide, involves inflammation of the mammary gland [1]. Mastitis affects the milk quality and production of the cow and may spread to other cows in the herd. The severity of the inflammation can be classified as subclinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactational status of the animal [4, 5].

Over 135 different organisms have been identified as the causative agents of bovine mastitis, including bacteria, viruses, mycoplasma, yeasts and algae [10]. In the present study, most of the mastitis cases were caused by CoNS and *Streptococcus* spp. Similar results were obtained in Poland by Malinowski et al. [6] and in Finland by Taponen et al. [12].

In our study the CoNS belong among the organisms most commonly isolated from milk samples of dairy cows with subclinical mastitis as in other studies [1, 8, 9].

In the Netherlands, the prevalence of CoNS among bacterial isolates from milk samples increased from 16.2 % in 1999 to 42.2 % in 2004 for subclinical mastitis, and from 7.3 % to 14.1 % for clinical mastitis [10].

In New Zealand, as in the UK, *Str. uberis* was the most common pathogen associated with clinical mastitis, but in

Table 1. CMT scores related to average somatic cell counts 2120 (11 rejected) quarters

CMT Score	SCC	% of quarters	Interpretation*
N (Negative)	0—200 × 103	63.2	Healthy quarter
T (Trace)	200—400 × 103	23.3	Subclinical mastitis
1	400—1000 × 103	6.5	Subclinical mastitis
2	1 000—3000 × 103	4.6	Serious mastitis Infection
3	Over 3000 × 103	2.0	Serious mastitis infection

* — Jackson and Cockcroft [3]

Table 2. Prevalence of mastitis in the quarters of the herds

Herd	No. of examined cows	No. of examined quarters	Healthy quarters		Rejected quarters	Positive quarters		Infected quarters	
			n	%		n	%	n	%
A	226	904	484	60.7	4	416	46.2	312	34.7a
B	304	1216	862	66.2	7	354	29.3	227	18.8b
Total	**530**	**2120**	**1346**	**63.5**	**11**	**770**	**36.3**	**539**	**25.6**

a, b, c — values within the same column with different superscript letters differ significantly at $P < 0.05$

Table 3. Microorganisms isolated from mastitis in the herds

Isolated microorganisms	n	Subclinical		Subacute		Acute	
		A %	B %	A %	B %	A %	B %
Staph. aureus	41	9.8	4.9	31.7	17.1	22.0	14.6
Str. uberis	17	11.8	-	47.1	-	41.2	-
Str. agalactiae	62	14.5	6.5	38.7	11.3	22.6	6.5
Streptococcus spp.*	55	14.5	12.7	38.2	11.0	-	7.3
CONS*	141	35.5	23.4	19.9	12.1	5.0	4.3
CPS*	24	37.5	16.7	33.3	20.8	-	-
E. coli	36	44.4	25	19.4	11.1	-	-
Enterococcus spp.	75	62.7	37.3	12	20	-	-
Bacillus spp.	48	43.8	20.8	25	2.1	-	8.3
Enterobacter aerogenes	17	64.7	35.3	47.1	-	-	11.8
Other*	23	56.5	34.7	8.6	-	-	-
Total	**539**	**35.3**	**20.6**	**26.3**	**11.5**	**6.9**	**4.1**

n — number of isolated bacteria; Other* — *Proteus* spp., *Aerococuss* spp.; CPS* — *S. aureus, S. hyicus*; *Streptococcus* spp. * — *Str. faecalis, Str. dysgalactiae, Str. Suis*; CONS* — *S. haemolyticus, S. chromogenes, S. xylosus, S. epidermidis, S. warneri*

contrast to the situation in the UK, *E. coli mastitis* was rarely observed [7]. In Norway, neither *Str. uberis* nor *E. coli* were commonly found. Rather, *S. aureus* and *Str. dysgalactiae* were the most common causes of clinical mastitis there [17]. The main pathogen associated with clinical mastitis in the Netherlands were CPS followed by *Str. dysgalactiae, Str. uberis* and *E. coli* [10]. IMI caused by *Strep. dysgalactiae* and mycoplasma were of major concern in the USA [3].

Coagulase-positive staphylococci (CPS) are permanently present in dairy herds. In our study, *S. aureus* and *S. hyicus* were isolated from 4.5 % of the quarters with subacute and acute forms of mastitis. Similar results were obtained by Malinowski et al. [5], who found *Staph. aureus* in 5.1 % of the cases of clinical and subclinical mastitis in the western part of Poland. This is in accordance with the data from other countries [11, 13].

CONCLUSIONS

The results of our study showed that the average prevalence of mastitis in two dairy herds (A and B) situated in marginal parts of Slovakia reached 14.1 % and 30.1 %, respectively. The bacteria *Staph. aureus, Str. agalactiae* and *Str. uberis* were most frequently isolated from clinical mastitis. The CoNS were the pathogens most frequently isolated from subclinical mastitis cases. The pathogens Str. agalactiae and *Staph. aureus* played an important role in dairy herds in the marginal parts of Slovakia.

A successful mastitis control programme should focus on the management of dry and calving cows and heifers. A clean and comfortable environment, proper feeding and adequate supplementation of the diet with vitamins and trace elements are essential for maintaining good udder health.

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