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Distribution of Bacterial Pathogens Causing Mastitis in Dairy Cows

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Abstract*— *Background: *Breeding of dairy ruminants represents a significant proportion of market milk production in agricultural regions localized in Slovak and Czech Republic. Mastitis is one of the biggest problems of dairy producers in these regions and causes great losses in the livestock economy.* ***Aim****: The aim of this study was to evaluate occurrence and aetiology of mastitis in two dairy herds of cows situated in Slovakia as well as in two dairy herds in Czech Republic.* ***Methods:*** *From a two Czech dairy herds, 405 and 320 lactating cows of holstein and czech spotted breed were included in the study. The same number of Slovak dairy farms with 226 and 153 cows of holstein and slovak spotted breed were exanimated. All herds were housed in a loose housing barn and milked twice a day. The diagnosis of mastitis was performed based on clinical examination of the udder, macroscopic evaluation of milk, with the evaluation of California mastitis test (CMT) and bacteriological analysis of milk samples from each exanimated cow.* ***Results:*** *Subclinical forms of mastitis were the most frequent in all monitored Slovak and Czech dairy herds. In the Czech dairy farms was recorded 16.0% and 6.8% occurrence of subclinical as well as 4.2% and 1.2% acute forms of mastitis, respectively. From Slovak dairy herds, the occurrence of subclinical mastitis was 11.3% and 7.8% with a 3.7% and 2.6% frequency of acute forms of mastitis. In the Czech and Slovak farm, the most commonly isolated pathogens were bacteria Staphylococcus spp. as group of coagulase negative staphylococci (CNS) and Staph. aureus. The others isolates from Czech farms were Enterococcus faecalis, Streptococcus uberis and Corynebacterium amycolatum. The second most frequent in the Slovak dairy farms were Aerococcus viridans, Enterococcus faecalis, and E. coli.* ***Conclusion:*** *CNS were the most frequently isolated from mastitis cases in all monitored herds, however mastitis caused by the contagious pathogens Staph. aureus and Str. uberis is still a problem. It should be borne in mind that the effectiveness of generally established methods for reducing environmental mastitis in combination with the major pathogens of the mammary gland is usually limited due to their polyethological and multifactorial origin.*

***Keywords*—** *Dairy cows; Slovak Republic; Czech Republic; Milking, Staphylococci.*

I. INTRODUCTION

Inflammation of the mammary gland - 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. Its impact is on milk yield and the quality as well as the animal welfare.[1,2]

The disease is usually local but may become systemic, although rarely, in immunocompromised animals. The incidence of the disease varies with the age of the animal and the stage of lactation. Based on clinical symptoms, mastitis can be divided into three groups: Clinical mastitis, Sub-clinical mastitis, or Chronic mastitis. Only relatively few udder infections result in "clinical mastitis" in which the udder is noted to be abnormal and the quality of milk secreted is altered. Most mastitis occurs as a low grade infection, a subclinical state (SM), which affects 10-15% cows, increasing milk leucocyte content, reducing milk production and increasing milk bacterial content. These all contribute to reduced milk value as a food and in monetary terms.[3]

According to Pyörälä and Taponen[4], more than 120 different microorganisms are considered to cause mastitis. Bacteria are the most common causative factor, recognized in more than 95% of mastitis cases. Bacteria involved in bovine mastitis are broadly classified as either contagious or environmental pathogens based on their epidemiological association with the disease.

Contagious pathogens are those organisms transmitted from cow to cow where the primary reservoir harboring the pathogens is the cow. The predominant contagious pathogens involved in bovine mastitis are *Staphylococcus aureus, Str uberis, Str. dysgalactiae* and *Str. 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 CNS (included *Staphylococcus cohni, S. xylosus, S. warneri, S. haemolyticus, S. intermedius, S. equorum, S. hyicus, S. schleiferi, S. piscifermentas, S. haemolyticus, S. epidermidis, S. chromogenes* and *S. pasteuri*), streptococci (*Strep. equinus, Strep. mitis, Strep. salivarius, Strep. Saccharolyticus)* and enterococci (*Enterococcus faecalis, E. faecium* and *E. avium*).[5] However, these organisms have a profound importance in both human and veterinary medicine.[6]

The aim of this study was to evaluate occurrence and aetiology of mastitis in two dairy herds of cows localized in Slovakia as well as in two dairy farms in Czech Republic.

II. METHODOLOGY

Dairy Herds and Milking

Monitoring of mastitis was carried out in a two dairy farms localized in Moravian region of Czech Republic and two dairy

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farms localized in east of Slovakia. From a two Czech dairy herds, 405 and 320 lactating cows of holstein and czech spotted breed were exanimated. From a two Slovak dairy farms, 226 and 153 cows of holstein and slovak spotted breed were included in the study. All herds were housed in a loose housing barn and fed total mixed ration according to international standards[7] to meet the nutritional requirements of a 600 kg cow. The cows were milked twice daily in a milking parlour with pulsation ratio 60:40 at a rate of 52 c/min and termination was automatically signaled when the milk flow dropped to 0.2 l/min. Blanket dry cow therapy was implemented in all herds.

Examination of Mammary Health Status

A complex examination of the health status of the animals included clinical examination of the mammary gland, cytological examination the first portion of milk, CMT reaction with subsequent collecting of individual milk samples (mixed quarters' samples) for bacteriological examination, and subsequent cultivation and identification of pathogenic bacteria.

CMT Evaluation and Laboratory Analyses

According to the procedure by Jackson and Cockcroft[8], milk from every quarter was mixed with the reagent and the result was read as trace, score 1, 2, 3, 4 or negative depending on the gel formation in the milk sample.

Mixed quarters' samples were collected aseptically according to our previous study.[9] Then the 10 mL of milk samples from each lactating cow were transported on ice to the laboratory. From each sample, 0.01 mL of milk was cultured on Columbia Blood Agar Bass (Oxoid, England) with 5% of defibrinated ram blood and incubated for 48 h at 37oC; the plates were examined after 24 and 48 h of incubation. Selected isolates were further characterized. All isolates were recovered in pure culture from individual samples. Colonies of *Staphylococcus* spp. were tested for coagulase activity (Staphylo PK, Imuna Pharm, SR). Growth-confirmed colonies of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were further identified biochemically using the STAPHYtest 24 (Fig. 1), STREPTOtest 24, resp. ENTEROtest 24 (Erba-Lachema, CZ) and the software TNW Pro 7.0 (Erba-Lachema, CZ). Colonies morphologically compatible with *Trueperella.pyogenes* were subjected to a conventional phenotypic assay API Coryne strips (BioMe'rieux, France).

Statistical Analysis

The differences in the occurrence of mastitis and isolated pathogens among herds localized in Slovak and Czech Republic were statistically analyzed using Yates corrected Ch-square test. The level of significance was set at $p<0.05$.

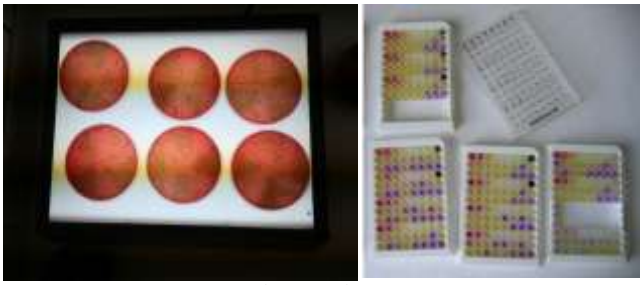

Figure 1. Laboratory identification of bacteria *Staphylococcus* spp. using biochemical STAPHY-test

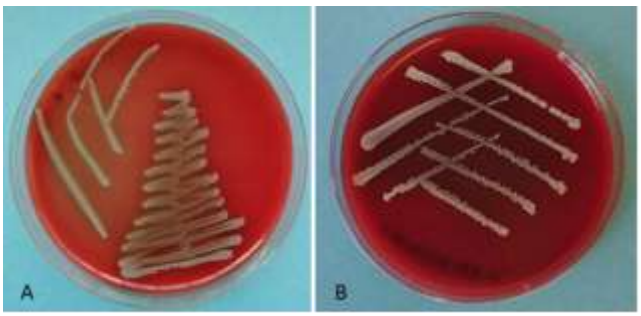

Figure 2. Mastitis pathogens after cultivation
Note: (A) *S. aureus*, (B) *S. epidermidis*

III. Results and Discussion

The table I shows the evaluation of the health status of the mammary gland of cows in monitored herds from Czech and Slovak Republic. The prevalence of mastitis in monitored Czech farms was 20.2% and 8.1%, respectively. From Slovak farms was prevalence of intramammary infection 15.0% and 10.5%, respectively.

Subclinical forms of mastitis were the most frequent in all monitored dairy herds. In the Czech dairy farms was recorded 16.0% and 6.8% occurrence of subclinical as well as 4.2% and 1.2% acute forms of mastitis, respectively. From Slovak dairy herds, the occurrence of subclinical mastitis was 11.3% and 7.8% with a 3.7% and 2.6% frequency of acute forms of mastitis (Table II).

TABLE I. Cows investigation from selected Czech and Slovak farms

Herd	No. of examined cows	Healthy quarters		Positive quarters CMT		Infected samples*	
		n	%	n	%	n	%
C1	405	1382	*85.3*^{a}	238	*14.7*^{a}	82	*20.2*^{b}
C2	320	1204	*94.0*^{b}	76	*6.0*^{b}	26	*8.1*^{a}
S1	226	780	*86.3*^{a}	124	*13.7*^{a}	34	*15.0*^{c}
S2	153	565	*92.3*^{b}	67	*10.9*^{c}	16	*10.5*^{a}

Note: n – number of exanimated quarters, CMT – California mastitis test, C1-C2 – selected Czech dairy farms, S1-S2 – selected Slovak dairy farms. Infected samples* - positive mixed quarters' samples, ^{a,b,c} – values within the same column with different superscript letters differ significantly at $P<0.05$.

Our results show, that CNS and *S. aureus* were the most common cause of mastitis in all monitored herds. From 160 positive samples, 47 isolates (29.3%) of CNS and 15 isolates of *S. aureus* were confirmed. From CNS, *S. xylosus*, *S. warneri*, *S. haemolyticus*, *S. chromogenes*, *S. schleiferi* and *S. piscifermentas* were the most commonly confirmed (Table II).

Staphylococcus spp. are one of the major etiological agents of mastitis in ruminants, where *Staph. aureus* is the most

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common pathogen of acute mastitis and CNS are causative agents of subclinical forms.[4,10]

TABLE II. Isolated microorganisms and forms of mastitis from monitored Czech and Slovak dairy herds

Pathogens	n	Subclinical (%)				Acute (%)			
		C1	C2	S1	S2	C1	C2	S1	S2
CNS	47	3.9	3.1	4.2	2.0	1.7		1.3	0.7
S. aureus	15	1.0	0.9		0.7	0.7	0.6		2.0
Coryn. amycolatum	7	1.2	0.6						
Str. uberis	18	2.5		1.3		0.7		0.8	
Str. bovis	6		0.6		2.0				0.7
Ent. faecalis	22	3.0	0.6	2.2		0.25			
E. coli	9	1.0		1.3		0.25		0.8	
A. viridans	11	0.5		1.8	3.3				
T. pyogenes	3	0.5				0.25			
Str. equinus	3		0.9						
Ser. marcescens	5	1.2							
Mixed infection	14	1.2		1.8		0.7	0.6	0.8	
Total	**160**	**16.0^{b}**	**6.8^{a}**	**11.3^{c}**	**7.8^{a,c}**	**4.2^{a}**	**1.2^{b}**	**3.7^{a}**	**2.6^{b}**

Note: n – number of positive mixed milk samples, C1-C2 – selected Czech dairy farms, S1-S2 – selected Slovak dairy farms, Mixed infections – infections caused two or more pathogens, ^{a,b,c} – values in the same row with different superscript letters differ significantly at P<0.05.

In recent years CNS have been identified as a source of clinical and subclinical mastitis[3,4] and rising cell counts, and they are known as an emerging mastitis pathogen.[11] They commonly colonize the teat skin, teat end and teat canal. Increased bulk tank levels of CNS may result from poor post-milking teat disinfection or from poor teat skin condition.[6]

Infection by CNS is not as pathogenic as other principal mastitis pathogens, and the infection is mostly subclinical. CNS can however increase SCC, leading to decreased milk quality. It can also damage the udder tissue leading to decreased milk production. The predominant species of CNS are *S. chromogenes* which affects nulliparous and primiparous cows, and *S. simulans*, which is more frequently isolated in older cows. The primiparous cows contract the infection during the time surrounding parturition, but the older cows contract the infection in late lactation.[4]

In a figure 3 is a comparison of individual mastitis pathogens isolated from mixed milk samples in farms from Czech and Slovak Republic. Apparently the most common pathogens were CNS, *Str. uberis*, *E. faecalis* and *Aerococcus viridans* from all monitored herds. The highest prevalence of CNS and *S. aureus* was observed in Slovak monitored herds. On the other hand, the highest incidence of *S. uberis* and *Ent. faecalis* was recorded in Czech herds.

In Norway 10-20% of acute clinical cases of mastitis is caused by *Streptococcus dysgalactiae* and *Str. uberis*. The bacteria can also cause subclinical cases of mastitis with very high SCC, but is easier to treat compared to *S. aureus*. It is considered as both a contagious and an environmental pathogen. Reservoirs include infected udders, manure, and other organic matter, including bedding. The presence of *Str. dysgalactiae* and *S. uberis* in milk samples from many cows is a sign of high infective pressure and/or that the milking procedure is too much of a strain on the udder. When many cows in a herd is infected with *Str. dysgalactiae*, it is important to make good routines to prevent the spread of disease.[12]

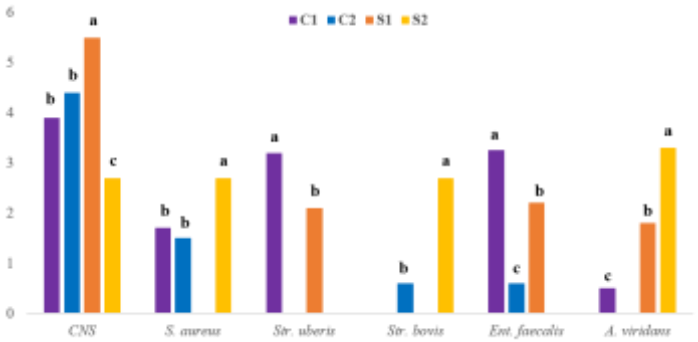

Figure 3. Occurrence and comparison of individual pathogens from infected cows
Note: C1-C2 – monitored Czech dairy farms, S1-S2 – monitored Slovak dairy farms, ^{a,b,c} – values above the column with different superscript letters differ significantly at P<0.05.

IV. Conclusion

The occurrence of mastitis varied in monitored dairy herds situated in Czech and Slovak Republic. Subclinical forms of mastitis were the most frequent in all dairy herds and bacteria *Staphylococcus* spp. (mainly CNS and *S. aureus*), *Streptococcus* spp., *E. faecalis*, and *Aerococcus viridans* were the most isolated from infected milk samples. From the point of view of the epidemiological importance, the highly contagious bacteria (*S. aureus* and *S. uberis*) 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.

It should be borne in mind that the effectiveness of generally established methods for reducing environmental mastitis in combination with the major pathogens of the mammary gland is usually limited due to their polyethological and multifactorial origin.

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