

MASTITIS PATHOGENS IN MILK OF DAIRY COWS IN SLOVAKIA

SH. E. IDRIS^{1*}, V. FOLTYS², V. TANČIN^{1,2}, K. KIRCHNEROVÁ², K. ZAUJEC²

¹Slovak University of Agriculture in Nitra, Slovak Republic

²Animal Production Research Centre Nitra, Slovak Republic

ABSTRACT

Mastitis, an inflammation of the mammary gland, is one of the most costly and complex diseases of the dairy cows. This study was done to evaluate the occurrence of mastitis pathogens in milk samples from cows with problematic udder health. Samples of milk for bacteriology were taken from dairy cows in an around Nitra region, Slovakia. For this purpose, the samples from udder quarters were cultured and bacteriologically evaluated. From 390 samples 73.85 % of positive samples were found. The predominant bacterial isolates were Coagulase negative staphylococci (17.95 %), followed by *Escherichia coli* (12.82 %), *Staphylococcus aureus* (9.74 %), *Bacillus* spp. (6.41 %), yeasts (5.64 %), *Streptococcus uberis* (4.1 %), *Staphylococcus epidermidis* (3.59 %), *Pseudomonas aerogenes* (3.33 %), others (bacteria and mould) (3.33 %), *Enterococcus* spp. (3.08 %), *Streptococcus agalactiae* (1.45 %), *Corynebacterium* spp. (1.28 %) and *Staphylococcus chromogenes* (1.03 %). In conclusion, high percentage of positive samples and relatively high occurrence of environmental microorganisms were identified in milk samples indicating the problem with the hygiene of the udder and environment in examined farms.

Key words: mastitis; milk bacteriology; dairy cows

INTRODUCTION

Mastitis can be considered as welfare, food safety and economic problem. Mastitis can cause chemical and bacteriological changes in milk and pathological changes in the mammary gland of the udder (Sharma, 2007). Somatic cell counts (SCCs) mean the number of cells in milk (in the case of mastitis there are mainly white blood cells as an immune response of mammary gland) (Sarıkaya *et al.*, 2006) and can indicate intramammary infection (IMI) when elevated (Reksen *et al.*, 2008). SCC is used as a diagnostic tool to monitor subclinical mastitis in dairy herds worldwide (Schukken *et al.*, 2003).

In Slovakia, the problem of environmental mastitis has gradually increased since year 2000. The prevalent pathogens causing mastitis are *Streptococcus uberis*, Coagulase negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, and the family of *Enterobacteriaceae* (Vasil', 2005). Milk

products are influenced by milk quality related to consumer demands (Kubicová and Dobák, 2012).

The most important major pathogens involved in bovine mastitis worldwide are *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Escherichia coli* and *Klebsiella* spp. (Olde Riekerink *et al.*, 2008). The impact of CNS is increasing (Pyörälä and Taponen, 2009), probably because prevalence of major pathogens is decreasing (Sampimon *et al.*, 2009). *Strep. agalactiae* and *Staph. aureus* are considered to be contagious (Barkema *et al.*, 2009), but environmental *Staph. aureus* mastitis may also occur (Zadoks *et al.*, 2002). *E. coli* and *Klebsiella* spp. have mainly an environmental origin (Munoz *et al.*, 2007). Other pathogens have both routes of infection. *Strep. uberis* IMI (intramammary infection) originates mainly from the environment (Pullinger *et al.*, 2006), but can also behave contagious (Zadoks *et al.*, 2003). *Strep. dysgalactiae* behaves intermediate between contagious and environmental transmission (Baseggio *et al.*, 1997).

*Correspondence: E-mail: sharafdm@yahoo.com
Sharaf Eldeen Idriss, Dept. of Evaluations and Processing of Animal Products,
Faculty of Biotechnology and Food Science, Slovak University of Agriculture,
Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
Tel.: +421 907 055 905

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For CNS, both environmental and contagious IMI occur (Taponen *et al.*, 2008).

Most of the intra-mammary infections arise during the process of milking or within 2 hours after it, i.e. to the time when the teat canal is fully closed. Tančin *et al.* (2006) described microbial contamination before and after preparation of the udder for milking. The aim of the study was to found out the microbiological contamination of raw milk by pathogens causing mastitis in milk of dairy cows.

MATERIAL AND METHODS

The study was conducted during the period from 2010-2012 in a surroundings Nitra region in Slovakia. A total of 390 milk samples were collected from dairy cows at some different small holder dairy farms, and pathogenic bacteria were examined. The samples were collected from farms with high bulk tank SCC and

consequently from cows with possible problems with udder health.

Milk sample collection and laboratory analysis

After a quarter had been cleaned up by removing any possible dirt and washed with tap water, the teat end was dried and swabbed with cotton soaked in 70 % ethyl alcohol. Approximately 100 ml of milk was collected aseptically into sterile bottles, after discarding the first 3 milking streams. Milk samples from each quarter were transported to the Laboratory of Animal Production Research Center in an ice cooled box at 4 °C and analysed immediately (max. 4 h after collection) either for identification of the clinical mastitis pathogen or to determine the reason for an increased somatic cell count (SCC). The milk samples were investigated for pathogenic mastitis according to a valid procedure of IDF (Bulletin, No.132, 1981).

Statistics: Statistical evaluation of the data was done using Excel program.

Table 1: Proportion of bacterial strains identified by complex examinations of milk from dairy cows within the period of 2010-2012 in Slovakia

| Major mastitis pathogens | Year of examination | | | | | | Proportion of pathogenic | |
|--------------------------------------|---------------------|-------|----------------|--------|----------------|-------|--------------------------|-------|
| | 2010 | | 2011 | | 2012 | | | |
| | n ₁ | % | n ₁ | % | n ₁ | % | n ₂ | % |
| Contagious pathogens | | | | | | | | |
| <i>Staphylococcus aureus</i> | 14 | 16.47 | 21 | 10.82 | 3 | 2.70 | 38 | 9.74 |
| <i>Streptococcus agalactiae</i> | 1 | 1.18 | 5 | 2.58 | 0 | 0 | 6 | 1.54 |
| Environmental pathogens | | | | | | | | |
| <i>Streptococcus uberis</i> | 4 | 4.71 | 7 | 3.61 | 5 | 4.50 | 16 | 4.10 |
| <i>Escherichia coli (E. coli)</i> | 5 | 5.88 | 23 | 11.86 | 22 | 19.82 | 50 | 12.82 |
| <i>Enterococcus spp.</i> | 0 | 0.00 | 6 | 3.09 | 6 | 5.41 | 12 | 3.08 |
| <i>Bacillus spp.</i> | 13 | 15.29 | 6 | 3.09 | 6 | 5.41 | 25 | 6.41 |
| Minor mastitis pathogens | | | | | | | | |
| <i>Corynebacterium pyogenes</i> | 5 | 5.88 | 0 | 0.00 | 0 | 0 | 5 | 1.28 |
| Coagulase-negative staphylococci | 7 | 8.24 | 33 | 17.01 | 30 | 27.03 | 70 | 17.95 |
| <i>Pseudomonas aeruginosa</i> | 0 | 0.00 | 13 | 6.70 | 0 | 0.00 | 13 | 3.33 |
| <i>Staphylococcus epidermidis</i> | 4 | 4.71 | 6 | 3.09 | 4 | 3.60 | 14 | 3.59 |
| <i>Staphylococcus chromogenes</i> | 4 | 4.71 | 0 | 0.00 | 0 | 0.00 | 4 | 1.03 |
| Yeasts | 1 | 1.18 | 2 | 1.03 | 19 | 17.12 | 22 | 5.64 |
| Others | 3 | 3.53 | 8 | 4.12 | 2 | 1.80 | 13 | 3.33 |
| Total of infected dairy cow quarters | 61 | 71.76 | 130 | 67.01 | 97 | 87.39 | 288 | 73.85 |
| Total of non-infected cow quarters | 24 | 28.24 | 64 | 32.99 | 14 | 12.61 | 102 | 26.15 |
| No. of dairy cow in the herd | 85 | 100.0 | 194 | 100.00 | 111 | 100.0 | 390 | 100.0 |

n₁ = number of examined dairy cows, n₂ = total number of pathogens, % = the percentage of the number of examined dairy cows
Others = (different types of bacteria and mold)

RESULTS AND DISCUSSION

In Table 1, proportions of bacterial strains identified by complex examination in dairy cows milk are presented. Positive results (infected quarters) were found in 288 samples (73.8 % of the total number of samples) depending on the year of the study. The proportion of bacteriologically negative samples (non-infected quarters) was 26.2 % (102 samples) (and also the effect of year was observed, as shown in Table 1).

Of these 288 isolates, CNS was the most common prevalent in 70 isolates (17.95 %), followed by *E. coli* 50 (12.82 %), *Staph. aureus* 38 (9.74 %), *Bacillus* spp. 25 (6.41 %), yeast 22 (5.64 %), *Strep. uberis* 16 (4.1 %), *Staph. epidermidis* 14 (3.59 %), *Pseudomonas* spp. 13 (3.33 %), others (mixed bacterial and mould) 13 (3.33 %), *Enterococcus* spp. 12 (3.08 %), *Strep. agalactiae* 6 (1.54 %) and *Corynebacterium* spp. 5 (1.28 %) isolates (Table 1). Infections likely caused by *Strep. dysgalactiae* and *Arcanobacterium* spp. were not occurring.

The highest occurrence of intramammary infections in year 2010 was caused by *Staph. aureus* 16.47%, followed by *Bacillus* spp. 15.29 %, CNS 8.24 %, *E. coli* 5.88 %, *Strep. uberis* 4.71 % and *Corynebacterium* spp. 5.88 % which hasn't occurred at the second and third years of study. While in 2011 the occurrence of CNS was 17.01 %, followed by *E. coli* 11.86 %, *Staph. aureus* 10.82 %, *Pseudomonas aeruginosa* 13.7 % which has only been detected in this year, and *Strep. uberis* 3.61 %. Whoever, in year 2012 only 14 dairy cows (12.16 %) was free from microorganism agents of mastitis. The most of the milk contamination was caused by CNS 27.03 %, *E. coli* 19.82 % and yeasts 17.03 %, while only 2.7 % by *Staph. aureus*, as is shown in Table 1.

Higher incidence of udder infections caused by pathogenic bacteria has been recorded by Ghazi and Niar (2006), and Fandrejewska (1993): 81.4 %, 66.8 % and 65.5 %, respectively. These results are similar to those in our study, where percentage of positive samples reached 73.85 %. Lower percentage of infected milk samples was published by Wilson *et al.* (1997) at the level of 48.5 %. The percentage of culture-negative samples in Netherland has been determined to be approximately 25 % (Barkema *et al.*, 1998), which corresponds to our observation (26.15 %).

In our study, the most frequent bacterial isolate has been found CNS 24.3 % (70 out of 288). We could also found out the increase in CNS occurrence during the study period. Coagulase-negative *Staphylococcus* spp. was isolated from 12.7 to 17.5 % by Makovec and Ruegg (2003). From the study performed on 20 conventional and 20 organic dairy farms, the prevalence of CNS IMI was 14 % on conventional farms and 17 % on organic farms (Pol and Ruegg, 2007). Last mentioned authors

also revealed CNS in 38 % and 30 % of milk samples on conventional and organic farms, respectively. In the study from Germany, 35 % of quarters with subclinical mastitis was caused by CNS (Tenhagen *et al.*, 2006). In the study carried out in the US and Canada, 15 % of new IMIs post-partum were due to CNS (Dingwell *et al.*, 2004). Among 77,051 routine mastitis samples submitted to laboratories in Finland during 2004-2006, CNS were the most frequently isolated bacteria in samples from clinical (18 %) and subclinical (24 %) mastitis cases (Koivula *et al.*, 2007).

Foltys and Kirchnerová (2005) found that the incidence of infections caused by *Staph. aureus* in 2001-2002 decreased from 29.30 to 10.30 %, respectively. Those results are similar to our findings. We found out only 2.7 % occurrence of *Staph. aureus* in 2012 indicating the improvement of the situation with contagious mastitis in dairy practice. There were also published reductions of *Staph. aureus* from 17.7 % in year 1997 to 9.7 % in year 2001 (Makovec and Ruegg, 2003).

E. coli and *Strep. agalactiae* were increased from 15.50 % to 28.20 % and 15.0 % to 20.40 % in 2003-2004, respectively (Foltys and Kirchnerová, 2005). The incidence of infections caused by *E. coli* is very difficult to eliminate in the environment where dairy cows are living. In our study incidence of *E. coli* mastitis was quite high and it superseded streptococcal mastitis. It could be due to poor hygiene conditions, as it infects the udder through teat canal (Sumathi *et al.*, 2008).

In our study incidence of mastitis due to yeast was found to be higher than *Strep. uberis* and *Strep. agalactiae*. Sporadic incidence of mastitis due to yeast has been reported by Ebrahimi and Nikookhah (2005). Stored antibiotics kept for repeated use may become contaminated with yeast and act as primary source of yeast and subsequent udder infection (Schalm, 1971). Tissue injury may also be helpful in establishing a mycotic mastitis. This obviously emphasizes the importance of strict aseptic measures in udder therapy with antibiotics.

CONCLUSIONS

Mastitis bacteriology, when used optimally as discussed, is an essential and cost effective tool in the ongoing control of mastitis and milk quality. Coagulase negative staphylococci (CNS) have been the most common bacteria identified in the whole survey. This means the impact of CNS is increasing, probably because prevalence of major pathogens is decreasing. Otherwise, the high frequency of CNS and *E. coli* occurrence indicated insufficient hygiene of housing and milking causing the risk of environmental mastitis.

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