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# COMPARISON OF ETIOLOGY OF ENVIRONMENTAL MASTITES IN TWO HERDS OF DAIRY COWS

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## ABSTRACT

The aim of our work was to compare the incidence, etiology and course of environmental mastitis in two herds of dairy cows with different technology of housing and milking. Observations were performed during 2005–2006 quarterly, using methods of clinical examination of the dairy cow udder, and milk cytological and bacteriological examinations. In both herds, the anti-mastitis measures were taken at the beginning of observation. In the herd with free housing of 112 dairy cows in full lactation, *Staphylococcus aureus* participated least in the intra-mammary infections from August 2005 to December 2006 (0.0 %–5.2 %), and *Streptococcus agalactiae* occurred only once (May 2006) in 2 dairy cows. There were more coagulase-negative staphylococci (1.8 %–36.1 %), and *Streptococcus uberis* occurred in the range from 29.1 % to 2.8 %. The course of intra-mammary infections was latent and sub-clinical. Anti-mastitis measures were efficient and ensured that in the second half of observation 2/3rd of dairy cows had SCC < 250 000.ml$^{-1}$ of milk. In the herd with stanchion housing of about 59 dairy cows in full lactation, *Staphylococcus aureus* participated the least (0.0–6.6 %) in the intra-mammary infections from August 2005 to July 2006, and coagulase-negative staphylococci were represented more (from 16.1 % to 2.2%), but *Streptococcus uberis* (0.0–18 %) had the highest count. Intra-mammary infections ran, above all, sub-clinically and latently, but later the occurrence of sub-acute and chronic mastitis increased. The introduced anti-mastitis measures were efficient – the number of dairy cows with SCC < 250,000.ml$^{-1}$ of milk increased from original 27.9 % to 80.4 % of dairy cows at the end of observation.

**Key words:** anti-mastitis measures, dairy cows, environmental mastitis, somatic cell count in milk, staphylococci, streptococci

## INTRODUCTION

In countries with developed dairy industry the problem of reduction of mastitis incidence in dairy cows induced by contagious pathogenic bacteria (*Streptococcus agalactiae* and *Staphylococcus aureus*) has been successfully solved in the last decade. But relative solution of given problem gave rise to a more complicated problem – mastitis incidence induced by environmental pathogenic bacteria belonging to the great number of species and genera.

To the most frequent environmental pathogens of the dairy cow mammary gland belong: *Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli, Klebsiela spp., Enterobacter spp., Serratia spp., Pseudomonas spp.* and others. The most frequent cause of inflammation is bacteria *Streptococcus uberis* and *E. coli* (Todhunter et al., 1995; Leigh, 1999). Zadoks et al. (2001) reported that *Streptococcus uberis* is an extended etiological agent of mastitis, above all in the large herds of dairy cows. High incidence of mastitis of this etiology was recorded by Barkema et al. (1998) in the Netherlands, Hillerton et al. (1993) in England and Scotland. Later, *Streptococcus uberis* was recorded as a significant agent of sub-clinical and clinical mastitis in New Zealand (McDougal, 1998; McDougal et al., 2004). A high contribution of *S. uberis* on the incidence of mastitis in dairy cows in the USA was reported by Smith and Hogan (1993), then by Hogan et al. (1989) and later by Rossitto et al. (2002). In Denmark, Zadoks et al. (2003) pointed to the problem of environmental mastitis. The capability to acquire a resistance against the effect of antibiotics and

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some disinfecting agents (especially on the basis of active iodine) contributes to the mass spreading of mastitis in dairy cows induced by *Streptococcus uberis*. In some countries, *Staphylococcus sp.* has been found to supplement the etiology of environmental mastitis. Finnish authors report that as much as a half of the isolated bacteria at mastitis are coagulase–negative staphylococci (Myllys et al., 1998; Pitkälä et al., 2004). Almost the same results under different breeding conditions were recorded by Sølvestød a Østerås (2001) in Norway. Vasiľ (2005) stated that the problem of gradual increase in environmental mastitis incidence under Slovak conditions, and mainly it is the case of udder inflammation, caused by *Streptococcous uberis,* coagulase–negative staphylococci, *Escherichia coli,* and cases induced by *Streptococcus dysgalactiae,* and bacteria of the family *Enterobacteriaceae*.

The aim of the work was to investigate the incidence, etiology, and course of environmental mastitis using clinical, cytological, and microbiological methods in two herds of dairy cows with different technology of breeding and milking after introduction of anti-mastitis measures.

## MATERIAL AND METHODS

Investigation of the incidence and course of mastitis in dairy cows induced by environmental pathogenic bacteria was carried out during 2005–2006 in two herds of dairy cows with different technology of housing and milking.

### Herds of dairy cows

The first herd consisting of about 112 lactating dairy cows (Black Pied and Slovak Pied cattle) was housed in the reconstructed stable with free box housing (straw bedding) and milking was performed twice a day in the milking parlour with milking equipment Westfalia tandem 2 x 4 (Bönen, Germany). Annual culling in the herd ranged up to 20 % and the herd was supplemented with high-pregnant heifers. The hygienic programme of milking was performed partially and incorrectly. Functional correctness of the milking equipment was controlled once a year. Following shortcomings were found: bedding was exchanged irregularly, cattle runs were not fixed, and at the time of unfavourable climatic conditions they became muddy; the hygienic programme for milking was not properly kept (udder washing with not very warm water, about 10 dairy cows were washed with the same cloth and about 12 dairy cows were towelled with one cloth (dipping of the teat peaks was not correct, often a minute after milking). The control of the correct function of milking equipment was performed irregularly. Cleaning and disinfection of housing premises was carried out once a year.

The second herd with about 59 dairy cows in full lactation (Pinzgau cattle) was housed in the production stable on the middle long stalls with bedding (sawdust, straw), chain-tied, milked by a milking equipment DeLaval MU210D (Tumba, Sweden) into the pipeline on the stall. During drying, parturition, and increasing milk flow about 20 dairy cows were housed in the delivery pen (separately on 22 middle-long stalls with straw bedding in the reconstructed cow-house K 98 with stanchion housing). The herd was supplemented with high-pregnant heifers. Annual culling ranged up to 19 %. The analysis of unfavourable state revealed following shortcomings: sawdust was used for bedding, housing premises were wet, the track to grazing was not fixed and was muddy; the hygienic programme for milking was not properly kept (insufficient preparation of dairy cows for milking – about 15 dairy cows were washed with the same cloth and about 8 dairy cows were towelled with one cloth). The control of the correct function of milking equipment was performed irregularly and there was incorrectness in daily, weekly and monthly cleaning and disinfection of milking equipment. Housing premises were cleaned and disinfected once a year.

### Anamnesis and examinations

The anamnestic data on the health status of dairy cows and correctness of application of technological procedures were obtained regularly during the whole experiment. A complex examination of the health status of the dairy cow udders was performed quarterly, and it included clinical examination of the udder, estimation of the first spray of milk, milk examination by NK-test with subsequent milk sampling (mixed quarter samples) for cytological and bacteriological examination (IDF Bulletin, No. 211, 1987). Cultivation and identification of pathogenic bacteria were carried out according to the IDF Bulletin. Cytological examination was performed using the Fosomatic apparatus and according to the STN 57 0532 norm.

### Proposed measures

After performing the first complex examination of the dairy cow udders in both herds, we recommended the measures for elimination of the shortcomings found through anti-mastitis measures. These were aimed at ensuring correct procedures of the hygienic programme, regularity in the control of correct function of the milking equipment, control of its cleaning and disinfection as well as housing premises in the sense of valid norms. The measures and procedures for ensuring "laic diagnostics" of clinical cases of mastitis by milkers and zoo-technicians and their subsequent fast treatment were recommended. From the beginning of the investigation, every dairy cow entering the dry period was treated using the application

of antibiotic preparations after last milking in given lactation and this step was finished by dipping the teat ending into efficient disinfecting preparation.

## RESULTS AND DISCUSSION

In the herd with free housing and milking in the milking parlour (table 1) at the beginning of observation, the highest representation in the occurrence of intramammary infections had coagulase–negative staphylococci (21.4 %), then environmental pathogenic bacteria (11.9 %, of which *Streptococcus uberis* comprised 7.1%) and then *Staphylococcus aureus* (4.8%). *Streptococcus agalactiae* did not occur at first three examinations, but it was recorded in May 2006 in two dairy cows (those were cured by penicillin preparations), and after that it was not recorded to the end of observation. The effectiveness of taken measures also positively manifested in the incidence of udder infection caused by the bacterium *Staphylococcus aureus*. Its occurrence was first reduced to a half, but in May 2006 it returned to the original level (then 4 dairy cows were cured and 2 dairy cows were culled out of the breeding). At the next examination it was not recorded, but at the last examination 3 dairy cows were infected (of which two were cured and one was culled). The effectiveness of taken measures and therapy at the infections induced by environmental pathogenic bacteria did not manifest during the first five months of observation except for infections induced by the bacteria *Streptococcus uberis* and *Streptococcus dysgalactiae*. In the next period a positive effect of the measures appeared, when a pronounced decrease in the infections induced by *E. coli*, *Enterococcus* sp., *Proteus vulgaris* and *Bacillus* sp. was recorded, but at their expense a number of infections caused by the bacteria *Streptococcus uberis* and *Streptococcus dysgalactiae* increased. Cogulase–negative staphylococci (CNS) at first resisted the effect of the measures, and therapy was effective only in 69.2 % (27 cured dairy cows out of 39 treated) cases. As a result, their occurrence in December 2005 was 36.1%, however,

**Table 1** **Number of infected dairy cows by the complex examinations in the herd of dairy cows with free housing in the years 2005 and 2006**

| Mastitis agent (bacteria) | Year and month of examination | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2005 | | | | | | 2006 | | | | | |
| | September | | November | | February | | May | | July | | September | |
| | $^{6}$n | % | $^{6}$n | % | $^{6}$n | % | $^{6}$n | % | $^{6}$n | % | $^{6}$n | % |
| Mastitis agents– infectious | | | | | | | | | | | | |
| *Staphylococccus aureus* | 6 | 4.8 | 3 | 2.8 | 3 | 2.8 | 6 | 5.2 | 0 | 0 | 3 | 2.7 |
| *Streptococcus agalactiae* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.7 | 0 | 0 | 0 | 0 |
| Mastitis agents–environmental | | | | | | | | | | | | |
| *Streptococcus uberis* | 9 | 7.1 | 6 | 5.5 | 3 | 2.8 | 12 | 10.4 | 9 | 8.3 | 32 | 29.1 |
| *Streptococcus dysgalactiae* | 0 | 0 | 3 | 2.8 | 0 | 0 | 6 | 5.2 | 6 | 5.6 | 12 | 10.9 |
| *E. coli* | 3 | 2.4 | 1 | 0.9 | 5 | 4.7 | 5 | 4.3 | 0 | 0 | 0 | 0 |
| *Enterococcus sp.* | 2 | 1.6 | 6 | 5.6 | 7 | 6.6 | 0 | 0 | 0 | 0 | 2 | 1.8 |
| *Proteus vulgaris* | 1 | 0.8 | 1 | 0.9 | 9 | 8.5 | 3 | 2.6 | 4 | 3.7 | 2 | 1.8 |
| *Bacillus sp.* | 0 | 0 | 0 | 0 | 6 | 5.7 | 0 | 0 | 2 | 1.9 | 2 | 1.8 |
| Mastitis agents– minority | | | | | | | | | | | | |
| Coagulasa–negative staphylococci | 27 | 21.4 | 39 | 36.1 | 5 | 4.7 | 16 | 13.9 | 13 | 12 | 2 | 1.8 |
| *Arcanobacterium pyogenes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2.8 | 0 | 0 |
| *Rhodococcus sp.* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.8 |
| Number of infected dairy cows | 48 | 3.1 | 59 | 54.6 | 38 | 35.8 | 50 | 43.5 | 37 | 34.3 | 57 | 51.8 |
| Number of uninfected dairy cows | 78 | 1.9 | 49 | 45.4 | 68 | 64.2 | 65 | 56.5 | 71 | 65.7 | 53 | 48.2 |
| Total number of dairy cows | 126 | | 108 | | 106 | | 115 | | 108 | | 110 | |

**Table 2** **Results of clinical examinations of the mammary gland and milk samples examination by NK–test in the herd of dairy cows with free housing in the years 2005 and 2006**

| Month | Number of dairy cows | Mastitis | | | | | NK-test | |
|---|---|---|---|---|---|---|---|---|
| | | Clinically apparent mastitis | | | | | | |
| | | Acute | Subacute and chronic mastitis together | Subclinical | Latent | bacterial | | |
| | [1]n | [3]% | [3]% | [3]% | [3]% | % | [2]np | [3]% |
| September* | 126 | 1.6 | 2.4 | 32.5 | 1.6 | 27.0 | 80 | 64.4 |
| November* | 108 | 0.9 | 1.8 | 24.1 | 16.7 | 27.8 | 62 | 57.4 |
| February** | 106 | 1.9 | 6.6 | 16.0 | 11.3 | 18.9 | 48 | 45.3 |
| May** | 115 | 0.9 | 5.2 | 20.9 | 16.5 | 14.8 | 59 | 51.3 |
| July** | 108 | 0 | 1.9 | 18.5 | 13.9 | 11.1 | 40 | 37 |
| November** | 110 | 1.8 | 7.3 | 24.5 | 18.2 | 10 | 66 | 60 |

* - year 2005; ** – year 2006; [1]n – number of examined dairy cows; [2]np – positive number ; [3] % – % of [1]n ;

in the next period a decrease in their incidence was recorded and at the end of observation were present only in two dairy cows (1.8 %). *Arcanobacterium pyogenes* was recorded only once in July 2006 in 3 dairy cows (2.8 %) and bacteria *Rodococcus* sp. also once at the end of observation in 2 dairy cows (1.8 %).

The results of clinical examination of the mammary gland of dairy cows and examination of milk by NK–test in the herd with free housing in 2005 and 2006 are presented in table 2. The proportion of dairy cows with clinically apparent mastitis, caused by bacterial agents, was during observation about 9.3 % and the occurrence of dairy cows with acute mastitis was minimal, but dairy cows with sub-acute and chronic mastitis occurred more often and variably (ranging from 1.8 % to 7.3 %). Regarding to the forms of mastitis running latently we recorded most dairy cows with sub-clinical mastitis (from 16.0 % to 32.5 %), less with abacterial mastitis (from 10.0 % to 27.8%), and the least dairy cows with latent mastitis (from 1.6 % to 18.2 %).

In table 3, the results of bacteriological and cytological examination of milk samples of the

**Table 3** **Results of bacteriological and cytological examinations of milk samples in dairy cows with free housing during monitored period**

| Month | Bacteriological examination of individual milk samples | | | Somatic cell content in 1 ml milk in individual milk samples (SCC in $10^3$.ml$^{-1}$) | | | [4]BT SCC (in $10^3$.ml$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | | | >250 | 250 - 500 | < 500 | |
| | [1]n | [2]np | [3]% | [3]% | [3]% | [3]% | |
| September* | 126 | 48 | 38.1 | 37.0 | 37.3 | 25.7 | 576 |
| November* | 108 | 59 | 54.6 | 58.3 | 21.3 | 20.4 | 511 |
| February** | 106 | 38 | 35.4 | 76.4 | 4.7 | 18.5 | 223 |
| May** | 115 | 50 | 43.7 | 66.5 | 13.7 | 19.7 | 286 |
| July** | 108 | 37 | 34.3 | 68.9 | 4.6 | 36.6 | 508 |
| November** | 110 | 57 | 51.8 | 66.1 | 6.4 | 26.5 | 268 |

* - year 2005 ; ** – year 2006; [1]n – examined number of samples ; [2]np – number of positive samples; [3] % – % of [1]n ; [4]BT SCC (in $10^3$.ml$^{1}$) – bulk tank somatic cell content in 1 ml milk (in $10^3$.ml$^{1}$)

dairy cow herd with free housing are shown. The results indicated that the content of somatic cells in the bulk tank milk sample (BTSCC) reflected the incidence of mastitis, and also the somatic cell content in individual milk samples corresponded with the health status of the dairy cow udders and the number of dairy cows with infected udder.

In the herd with stanchion housing (table 4), at the beginning of observation the environmentally pathogenic bacteria participated in intra-mammary infections most (in 24.6 %, of which *Streptococcus uberis* accounted for 18 %) followed by coagulase–negative staphylococci (9.8 %), and the least represented species was *Staphylococcus aureus* (6.6%). *Streptococcus agalactiae* was not detected in this herd. The breeder introduced ordered anti-mastitis measures gradually from September to December 2005. Their effect manifested positively especially in the occurrence of *Staphylococcus aureus*, which infected one dairy cow at the second examination (the cow was culled), after that it was not reported. The slowness at the introduction of the anti-mastitis measures resulted in the increase in the number of dairy cows with mastitis induced by the environmental bacterial agents in December 2005 to 35.5 % with a high representation of the bacteria *Enterococcus* sp. (17.7%), which served as an accompanying sign of diarrhoeic diseases in the herd (due to dietetic mistakes in nutrition). The representation of *Streptococcus uberis* (8.1 %) and *E. coli* (8.1) was high as well, while *Proteus vulgaris* occurred randomly (1.6 %) with latent course. Both the application of adopted anti-mastitis measures and therapy of clinical cases were effective in reducing the number of infected dairy cows except for those infected with bacteria *Proteus vulgaris*, where the number of infected dairy cows first increased to 3.5 %, and later up to 7.5 %. The number of dairy cows with intra-mammary infection induced by coagulase–negative staphylococci was as follows: 9.8 % at the beginning of observation, 16.1% at the second examination, 3.4 % at the third examination, and 7.5 % at the end, i.e. the effect of adopted measures was not pronounced.

Clinical examination of the mammary gland in the herd with stanchion housing of dairy cows during observation (table 5) revealed only few clinically apparent forms of mastitis caused by bacteria. Acute mastitis at the first examination was 1.6%, at the second 4.8 %, and at the third 3.4 %, while at the end of the observation no mastitis with acute course was recorded. The number of dairy cows with sub-acute and chronic mastitis gradually increased from1.6 % at the beginning to 9.4 % of dairy cows at the end of observation. Clinical examination revealed more sub-clinical inflammations of the udder than latent infections, while a marked portion of the clinical findings represented abacterial mastitis. Their occurrence had a decreasing tendency from 31.1 % at the beginning to 3.8 % of dairy cows at the end of the experiment.

**Table 4** **Number of infected dairy cows by complex examinations in the herd of dairy cows with stanchion housing in the years 2005 and 2006**

| Mastitis agent (bacteria) | Year and month of examination | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2005 | | | | 2006 | | | |
| | August | | December | | March | | April | |
| | $^{5}$n | % | $^{5}$n | % | $^{5}$n | % | $^{5}$n | % |
| Mastitis agents– infectious | | | | | | | | |
| *Staphylococccus aureus* | 4 | 6.6 | 1 | 1.6 | 0 | 0.0 | 0 | 0.0 |
| Mastitis agents–environmental | | | | | | | | |
| *Streptococcus uberis* | 11 | 18.0 | 5 | 8.1 | 5 | 8.6 | 0 | 0.0 |
| *Streptococcus dysgalactiae* | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| *E. coli* | 1 | 1.6 | 5 | 8.1 | 1 | 1.7 | 1 | 1.9 |
| *Enterococcus sp.* | 3 | 5.0 | 11 | 17.7 | 4 | 6.9 | 0 | 0.0 |
| *Proteus vulgaris* | 0 | 0.0 | 1 | 1.6 | 2 | 3.5 | 4 | 7.5 |
| Mastitis agents– minority | | | | | | | | |
| Coagulasa–negative staphylococci | 6 | 9.8 | 10 | 16.1 | 2 | 3.4 | 4 | 7.5 |
| Number of infected dairy cows | 25 | 41.0 | 33 | 53.2 | 14 | 24.1 | 9 | 27.0 |
| Number of uninfected dairy cows | 36 | 59.0 | 29 | 46.8 | 44 | 75.9 | 44 | 83.0 |
| Total number of dairy cows | 61 | | 62 | | 58 | | 53 | |

**Table 5** **Results of clinical examination of the mammary gland and milk samples examination by NK–test in the herd of dairy cows with stanchion housing in the years 2005 and 2006**

| Month | Number of dairy cows | Mastitis | | | | | NK–test | |
|---|---|---|---|---|---|---|---|---|
| | | Clinically apparent mastitis | | Subclinical | Latent | Bacterial | | |
| | | Acute | Subacute and chronic mastitis together | | | | | |
| | [1]n | [3]% | [3]% | [3]% | [3]% | [3]% | [2]np | [3]% |
| August* | 61 | 1.6 | 1.6 | 37.7 | 0.0 | 31.1 | 44 | 72.1 |
| December* | 62 | 4.8 | 2.3 | 21.0 | 14.5 | 24.2 | 35 | 56.4 |
| March** | 58 | 3.4 | 6.9 | 10.3 | 3.4 | 8.6 | 20 | 34.5 |
| June ** | 53 | 0.0 | 9.4 | 5.7 | 5.7 | 3.8 | 11 | 20.8 |

* – year 2005; ** – year 2006; [1]n – number of examined dairy cows; [2]np – positive number; [3] % - % z [1]n – % of [1]n ;

Results of milk examination by NK–test at the individual examinations corresponded to the BTSCC (table 6), clinical status of the dairy cow udders (table 5), and the number of infected dairy cows in the herd (table 1). From the results of cytological examination and SCC determination (table 6) it has been found that the effect of anti-mastitis measures was manifested in the second half of the experiment.

Our results are in accordance with those reported by Green et al. (2002), Zadok et al, (2003) and Vasil' (2005) who aimed at specificities of prevention and inhibition as well as forms of manifestation of clinical symptoms, incidence, and course of environmental mastitis. These authors stated that new infections induced by environmental pathogenic bacteria arise more frequently during the dry period than during lactation. The most critical period for onset and manifestation of these infections is the 2 weeks before parturition, regardless whether dairy cows at drying were or were not treated with antibiotics. In the herds where the therapy is not applied during the dry period, the first 2 weeks of this period are critical. In the period of lactation the first 75 days are critical. 5 to 10 % of heifers are estimated to show infection induced by the environmental pathogenic bacteria during the period of parturition, and these infections will manifest with clinical mastitis after parturition. Environmental infections in dairy cows last shorter than those of the mammary gland induced by infectious agents. Approximately 60% of infections with environmental streptococci last less than 30 days, and 18% of these infections are changed into chronic status, while they persist more than 100 days (Zadok et al., 2003). In coliform infections about 40% of cases last less than 7 days and chronic infections are very sporadic.

**Table 6:** **Results of bacteriological and cytological examinations of milk samples in dairy cows with stanchion housing during monitored period**

| Month | Bacteriological examination of individual milk samples | | | Somatic cell content in 1 ml milk in individual milk samples (SCC in $10^3$.ml$^{-1}$) | | | [4]BT SCC (in $10^3$.ml$^{1}$) |
|---|---|---|---|---|---|---|---|
| | | | | < 250 | 250 - 500 | >500 | |
| | [1]n | [2]np | [3]% | [3]% | [3]% | [3] % | |
| August* | 61 | 25 | 41.0 | 27.9 | 27.9 | 44.3 | 763 |
| December* | 62 | 33 | 53.2 | 46.5 | 14.8 | 38.7 | 706 |
| March** | 58 | 14 | 24.1 | 70.7 | 6.9 | 19.0 | 516 |
| June** | 53 | 9 | 17.0 | 80.4 | 7.3 | 12.3 | 385 |

* – year 2005; ** – year 2006; [1]n – examined number of samples ; [2]np – number of positive samples; [3] % - % z [1]n – % of [1]n ;
[4]BT SCC (in $10^3$.ml$^{1}$) – bulk tank somatic cell content in 1 ml milk (in $10^3$.ml$^{1}$)

In environmental pathogens a spontaneous elimination of infection plays an important role and it is estimated that at the infections with environmental streptococci 38% cases are eliminated spontaneously and most at those with coliform bacteria. Clinical form of the disease occurs in 40 to 50% infections induced by environmental streptococci. Only 9% of these infections are accompanied with the symptoms of general disease. These diseases mostly run easily in the form of sub-acute, or acute catarrhal mastitis (Vasiľ, 2005), which was also confirmed in our case. Infections with coliform pathogens manifest in clinical mastitis in 80 % cases, and 10% cases of these mastitis manifest in serious form with per-acute course endangering the animal life. In other cases they run easily in the form of acute catarrhal mastitis. Clinical forms of the disease caused by environmental pathogens are concentrated to the beginning of lactation, 20% arise in the first 7 days after parturition (Green et al., 2002). 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. Microbial contamination before and after preparation of the udder for milking was described by Tančin et al. (2006).

From the viewpoint of potential risk for the human health, bacterial infections of the ruminant udder by *Staphylococcus* sp. (Foltys and Kirchnerová, 2005) appear to be very ~~important,~~ serious, which under certain conditions could produce enterotoxins. Staphylococcal enterotoxins are mainly produced by some strains of *Staphylococcus aureus* (Vasiľ et al., 2007), but also coagulase–positive staphylococci *S. intermedius* and *S. hyicus* (Bergdoll, 1990; Vasiľ et al., 2007). However, some strains of coagulase–negative staphylococci such as *S. cohnii, S. epidermidis, S. haemolyticus, S. xylosus* (Bautista et al., 1988), *S. chromogenes, S. warnei, S. sciuri, S. saprophyticus, S. lentus* (Valle et al., 1990) are also presented as enterotoxigenic ones. Almost all the presented strains of staphylococci are isolated from milk of ruminants in our herd, too. In the last years an increased frequency of the occurrence of coagulase-negative staphylococci has been recorded at the examination of milk samples in our herd of dairy cows and sheep (Vasiľ, 2005, Vasiľ et al., 2005).

The problem becomes complicated because mastitis induced by the environmental pathogenic bacteria and coagulase–negative staphylococci are also recorded in the herd of small ruminants, and they have common causes with cow herd (Tripathi, 2000), i.e. shortcomings in hygiene of housing, failure in the hygienic programme of milking, incorrect function of milking equipment, decrease in the general hygienic standard etc. (Tongeľ and Mihina, 1999; Dudríková, 2001; Vasiľ et al, 2005).

Methods successfully used for prevention and inhibition of mastitis, caused by contagious agents, are often ineffective at elimination of the environmental pathogenic bacteria (Schukken et al,, 1989; Gonzalez et al., 1990); this is confirmed by the presented results. Despite species heterogeneity of the pathogenic bacteria causing environmental mastitis, this group of pathogens has some common characteristics leading to their role at the onset of the inflammatory process in the mammary gland, and their understanding is a basic precondition for solving the problems caused by them.

## CONCLUSION

The results of investigation of the incidence, etiology and course of environmental mastitis in two herds of dairy cows have been obtained with technologies given below:

A.

In the herd with free housing of dairy cows and milking in the milking parlour the anti-mastitis measures manifested as follows:

- Measures were effective in reduction of intra-mammary infections caused by main (infectious) and side agents of mastitis; their effect against environmental pathogenic bacteria was limited.
- Occurrence of dairy cows with acute mastitis was pronouncedly reduced, as well as those with sub-acute and chronic forms, and most of the intra-mammary infections had a latent or sub-clinical course.
- Mistakes in the building design of technology were the cause of a relatively high number of dairy cows with abacterial mastitis.
- A high portion of dairy cows with the content of somatic cells to 250 000.ml$^{-1}$ milk testifies to a very favourable effect of anti-mastitis measures and applying fast therapy of clinical mastitis.

B.

In the heard with stanchion housing of dairy cows and milking on stall the anti-mastitis measures manifested as follows:

- Measures were effective in reduction of intra-mammary infections caused by main and environmental pathogenic bacteria, and at the same time they also reduced the occurrence of side pathogenic bacteria.
- Measures positively influenced the occurrence of dairy cows with acute mastitis only at the end of observation, they did not influence a gradual increase in the number of dairy cows with sub-acute and chronic mastitis, but they apparently reduced the number of dairy cows with latent, sub-clinical and abacterial mastitis.
- A high portion of dairy cows with the content of somatic cells to 250000.ml$^{-1}$ milk during the second year of observation testifies to the favourable effect of anti-mastitis measures on the general health status of the dairy cow mammary glands in the herd.

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## REFERENCES

BARKEMA, H. W. – SCHUKKEN, Y. H. – LAM, T.J.G.M. – BEIBOER, M. L. –WILMINK, H. – BENEDICTUS, G. – BRAND,.A.1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell count. In: *J. Dairy Sci*. vol. 81, 1998, no. 2, p. 411-419.

BERGDOLL, M.S.1990. Analytical Methods for *Staphylococcus aureus*. Int. *J. Food Microbiol.,* vol. 10, 1990, no. 2, p. 91 – 100.

DUDRÍKOVÁ, E. 2001. Surové ovčie mlieko a výrobky z neho z pohľadu legislatívy a technológie spracovania. (Raw sheep milk and products from it regarding legislation and technology of processing). In: *Slovenský veterinársky časasopis (Slovak Veterinary Journal)*, roč. 26, 2001, č. 5, s. 270-275.

FOLTYS, V. – KIRCHNEROVÁ, K.1999. Development of mastitis pathogens occurrence and their susceptibility to antibiotics in basic production of milk. In: *Journal of Farm Animal Science (Science works Research Institute of Animal Production, Nitra)*, vol. 38, 2005, p. 177 – 180

GONZALEZ, R.N. – JASPER, D.E.,– KRONLUND, N.C. – FARVER, T.B.– CULLOR, R.B.– BUSCHNELL, R.B. – DELLINGER, J.D. 1990. Clinical mastitis in two California dairy herds participating in contagious mastitis control programme. In: *J. Dairy Sci.*, vol. 73, 1990, no. 3, p. 648-660.

GREEN, M. J. – GREEN, L. E. –MEDLEY, G. F. – SCHUKKEN, Y. H. – BRADLEY, A. J. 2002. Influence of Dry Period Bacterial Intramammary Infection on Clinical Mastitis in Dairy Cows. In. *J. Dairy Sci.,* vol. 85 2002, no.9, p. 2589-2599.

HILLERTON, J.E.– SHEARN, M.P.F.– TEVENSON, R.M – LANDRIDGE, S –BOTH, J.M. 1993. Effect of pre-milking teat dipping on clinical mastitis on dairy farms in England. In: *J.Dairy Res*., vol. 60, 1993, no. 1, p. 31-41.

HOGAN, J.S., SMITH, K.L., HOBLET, K.H., SCHOENBERGER, P.S., TODHUNTER, D.A., HUESTON, W.D., FRITCHARD, D.E., BOWMAN, G.L., HEIDER, L.E., BROCKETT, B.L., CONRAD, H.R. 1989. Field survey of clinical mastitis in low somatic cell count herds. In: *J. Dairy Sci.,* vol. 72, 1989, no. 6, p. 1547-1556.

INTERNATIONAL DAIRY FEDERATION BULLETIN. 1981. Laboratory Methods for use in Mastitis Work. *Document No l32, IDF Brussels 1981*, 27 s.

INTERNATIONAL DAIRY FEDERATION BULLETIN. 1987. Bovine Mastitis – definition and Guidelines for diagnosis. *Document No. 211, IDF Brussels 1987*, 22 s.

LEIGH, J.A., 1999. *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis. In*: Vet. J.*, vol. 157, 1999, no. 2, p. 225-238.

MCDOUGALL, S., 1998. Efficacy of two antibiotic treatments in curing clinical and subclinical mastitis in lactating dairy cows. In: *NZ vet. J.,* vol. 46, 1998, no. 6, p.226-232.

MCDOUGALL, S. – PARKINSON, T.J. – LEYLAND, M. – ANNISS, F.M. – FENWICK, S.G. 2004. Duration of infection and Strain Variation in *Streptococcus uberis* Isolated from Cows Milk. In*: J. Dairy Sci.*, vol. 87, 2004, no. 7, p.2062-2072.

MYLLYS, V., K. ASPLUND, E. BROFELDT, V. HIRVELÄ-KOSKI, T. HONKANEN-BUZALSKI, J. JUNTTILA, L. KULKAS, O. MYLLYKANGAS, M. NISKANEN, H. SALONIEMI, M. SANDHOLM, AND SARANPÄÄ, T. 1989. Bovine mastitis in Finland in 1988 and 1995 - Changes in prevalence and antimicrobial resistance. In: *Acta Vet. Scand.*, vol. 39, 1989, no.1, p. 119–126.

PITKÄLÄ, A. – HAVERI, M. – PYÖRÄLÄ, S. – MYLLYS, V. – HONKANEN-BUZALSKI, T. 2004. Bovine Mastitis in Finland 2001- Prevalence, Distribution of Bacteria, and Antimicrobial Resistance. In: *J. Dairy Sci.*, vol. 87, 2004, no. 8, p.2433-2441.

ROSSITTO, P.V. – RUIZ,L – KIKUCHI, Y. – GLENN, K. – LUIZ, K. – WATTS, J.L. –CULLOR, J.S. 2002. Antibiotic Susceptibility Oat terns for Environmental Streptococci isolated from Bovine Mastitis in Central California Dairies. In: *J. Dairy Sci.*. vol. 85, 2002, no. 1, p. 132-138.

SCHUKKEN, Y.H. – GROMMERS, F.J. – VAN DE GEER, T. – BRAND, A. 1989. Incidence of clinical mastitis on forms with low somatic cell counts in bulk milk. In: *Vet. Rec*., vol. 125, 1989, no. 3, p. 60-63.

SMITH, K.L. – HOGAN, J.S. 1993. Environmental mastitis. In: *Vet. Clin. North Am.: Food Animal Pract.,* vol. 9, 1993, no. 3, p. 489-498

SØLVESTØD, L. – ØSTERĹS. O.:2001. The Norwegian survey of subclinical mastitis during. In: *Proc. 2nd Int. Symp. Mastitis and Milk Quality, BC Vancouver 2001,* p.126-130.

TANČIN V. – KIRCHNEROVÁ, K. – FOLTYS, V. – MAČUHOVA, L. – TANČINOVÁ, D. 2006. Mikrobiologická kontaminácia a počet somatických buniek vo vzorkach mlieka dojníc odobratých pred a po príprave dojníc na dojenie. (Microbial contamination and somatic cell count of bovine milk striped and after udder preparation for milking). In: *Slovak J. Anim. Sci.,* vol. 39, 2006, no. 4, p. 214-217.

TONGEL. P. – MIHINA, Š. (Research Institute of Animal Production, Nitra): The raising of milk quality by means of separate milking. : In: *Journal of Farm Animal Science (Science works Research Institute of Animal Production, Nitra),* vol. 32, 1999, p. 79-85.

TODHUNTER, D.A. – SMITH, K.L. –HOGAN, J.S. 1995. Environmental streptococcal intramammary infections of

the bovine mammary gland. In: *J. Dairy Sci.,* vol. 78, 1995, no. 11, p. 2366-2374.

TRIPATHI, B.N. 2000. Diseases of the mammary glands of goasts and sheep. In. *Vet. Bulletin,* vol. 70, 2000, no. 11, p.11,441-452.

STN 57 0532. Stanovenie počtu somatických buniek v mlieku (Determination of the number of somatic cell in milk) . Bratislava. SÚTN, 1994.

VALLE, J. – GOMEZ, L.E. – PIRIZ, S. – GOYACHE, J. – ORDEN, J.A. –VADILO, S. 1990. Enterotoxins production by Staphylococcal Isolated from Healthy Goats. In: *Applied and Environmental Microbiology,* vol. 56, 1990, no. 5, p.1323 – 1326.

VASIĽ, M.2005. Účinnosť kauzálnej a symptomatickej liečby environmentálnych mastitíd dojníc (The effect of causal and symptomatic treatment of environmental mastitis in dairy cows. *Poľnohospodárstvo ( Agriculture),* roč. 51, 2005, č. 4, s. 200-206.

VASIĽ, M. – EEČKO, J. – FOTTA, M.: Mikrobiologická zaťaženosť ovčieho mlieka od prvovýroby po jeho spracovanie **(**The microbiological load of sheep milk from primary production to its processing). *Poľnohospodárstvo (Agriculture*), roč. 51, 2005, č. 11, s. 593-601.

VASIĽ, M. ─ FOTTA, M. –ELEČKO, J. 2007. Produkcia enterotoxínov druhmi rodu *Staphylococcus* sp. izolovaných z ovčieho mlieka (Enterotoxin production by the species of *Staphylococcus* sp. isolated from sheep milk*). Slovak J. Anim. Sci.,* vol. 40., 2007, no. 1, p. 52 – 56.

ZADOKS, R.N. –ALLORE, H.G. – BARKEMA, H.W. – SAMPIMON, O.C. – GRÖHM, Y.T. –SCHUKKEN, Y.H. 2001. Analysis of an Outbreak of *Streptococcus uberis* Mastitis. In: *J. Dairy Sci.,* vol. 84, 2001, no. 3, p. 590-599.

ZADOKS, R.N. – GILLESPIE, B.E. –BARKEMA, H.W. – SAMPIMON, O.C. – OLIVER, P. – Y.H. SCHUKKEN, 2003. Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. In: *Epidemiol. Infect.,* vol. 130, 2003, no. 3, p. 335-349.

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