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ETIOLOGY, COURSE AND REDUCTION OF INCIDENCE OF ENVIRONMENTAL MASTITIS IN THE HERD OF DAIRY COWS

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ABSTRACT

The work was aimed at the observation of the incidence and course of environmental mastitis in the breeding with a mean number of 177 dairy cows free housed and milked in the milking parlour after introduction of anti-mastitis measures. During 6 months of application of preventive inhibitory methods the incidence of bacteriological positive dairy cows was reduced from 72.99 % to 6.74 %, and occurrence of dairy cows with clinically apparent form of mastitis decreased from 23.00 % to 0.56 %. The main etiological agents of intra-mammary infections were bacteria *Streptococcus sp., Arcanobacterium pyogenes* and coagulase–negative staphylococci. The overall efficacy of the preparation Synulox LC used for the treatment of intra-mammary infections ranged from 88.71 % to 96.15 %. *Streptococcus sp.* bacteria were 100% sensitive against combination of amoxycilin with clavulanat. *Arcanobacterium pyogenes* bacteria showed the sensitivity of 93.6 %, whilst the sensitivity of coagulase-negative staphylococci bacteria was at 90.0 %.

Key words: environmental mastitis, dairy cows, staphylococci, streptococci, *Arcanobacterium pyogenes,* anti-mastitis measures, mastitis treatment

INTRODUCTION

At present in Slovakia most of the breeders of dairy cows successfully have coped with the problem of mastitis induced by *Streptococcus agalactiae* and *Staphylococcus aureus*. However, despite the effort to ensure good care some breedings have failed to produce for a long time milk containing somatic cells in a pool sample lower than 400 000.ml^{-1} (Vasil', 2007). This problem occurs in most of the breedings mainly in spring season during wet summer weeks and at the beginning of autumn and is accompanied with a higher frequency of clinically apparent forms of mastitis. At bacteriological examination of milk from suspect cows, or secretion of the cow udder with clinical mastitis we can detect *Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli, Enterobacter sp., Arcanobacterium pyogenes, Klebsiela spp., Serratia spp., Pseudomonas spp.* and others, i.e. pathogenic bacteria easily surviving in the external environment belonging to a great group of environmental pathogens of the mammary gland of dairy cows.

In general, *Streptococcus uberis* and *Escherichia coli* are considered to be the most frequent agents of mastitis (Leigh, 1999). *Streptococcus uberis* is a widespread etiological agent of mastitis in numerous herds of dairy cows (Barkema et al., 1998; Zadoks et al., 2001). More authors in the Netherlands, England, and Scotland draw attention to a high incidence of mastitis of this etiology in the world. McDougall et al. (2004) determined *Streptococcus uberis* as significant agents of sub-clinical and clinical mastitis in the New Zealand. As well, a high portion of *Streptococcus uberis* in the incidence of mastitis in dairy cows was recorded in the USA (Rossitto et al., 2002). High incidence of the environmental mastitis in the Denmark is reported by Zadoks et al. (2003).

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Probably the reason of the high portion of *Streptococcus uberis* in the environmental mastitis of ruminants is a fact that it easily acquires a resistance not only against antibiotics, but also disinfectant preparations (also on the basis of active iodine). *Staphylococcus sp.* bacteria are also very significant in the etiology of mastitis. Pitkälä et al. (2004) reported that a half of the bacteria isolated at environmental mastitis in dairy cows in Finland are the coagulase-negative staphylococci.

In Slovakia the problem of environmental mastitis has gradually increased since 2000. First, there were mainly inflammations of the udder caused by the germs of *Streptococcous uberis, Escherichia coli* and bacteria from the family *Enterobacteriaceae*. Gradually the cases induced by coagulase-negative streptococci *Pseudomonas spp.*, but also bacteria *Proteus sp.*, and the incidence of mastitis induced by *Arcanobacterium pyogenes* and *Streptococcus dysgalactiae* have increased (Vasil', 2006). Mastitis of dairy cows induced by *Streptococcus dysgalactiae* is significant especially because they, through their way of spreading in the herd, are very close to contagious agents of mastitis. Infections caused by the bacterium *Arcanobacterium pyogenes* are often manifested by clinical mastitis. Most frequently they occur in dairy cows on pasture, or in heifers raised by pasture rearing before and after parturition, as well as in cows with udder injury either in free or in stanchion housing. Prognosis of mastitis caused by *Arcanobacterium pyogenes* is mostly unfavourable, antibiotic therapy is not very efficacious, injured quarter still shows chronic changes, its production ability is lower and in further period the changes are going deeper and it leads to atrophies. The sources of infections are mostly wounds, teat injures, prevalent abscesses, contaminated environment after parturition of cows infected by *Arcanobacterium pyogenes*, or inflammations of birth canals. Transmission of infections occurs, above all, by the contact of teats with contaminated environment, and very often also by flies, especially by *Hydrotaea irritans.* The incidence of mastitis induced by *Arcanobacterium pyogenes* is apparently connected with wet weather. The authors from England and Northern Europe termed them as „summer" mastitis (Menzies and Mackie, 2001). Etiology of these forms of mastitis besides *Arcanobacterium pyogenes* in a common bacteriological finding is also supplemented by other bacteria, e.g. *Staphylococcus aureus*, *Escherichia coli*, *Corynebacterium* sp. bacteria, and quite often at these mastitis coagulase–negative staphylococci or *Streptococcus uberis* are also isolated together with *Arcanobacterium pyogenes.*

The goal of this work was to observe the incidence, etiology and course of environmental mastitis and efficiency of the anti-mastitis measures for reduction of the level of contamination in dairy cows breeding at free housing and milking in the milking parlour.

MATERIAL AND METHODS

Observations in the herd of dairy cows

Observations were performed in the herd with a mean number of 177 dairy cows housed freely in 6 sections (about 30 cows) of the reconstructed cowshed (originally for 176 cows) with grates covering the preronic system of manure removal. Productive cowshed is connected through a corridor with a milking parlour equipped with milking equipment Boumatik 24. Culling in the herd was at 25 %. The herd was supplemented by its own high-pregnant heifers raised using pasture rearing.

Before beginning of the observation technological and operational imperfections were revealed. The housing sections (about 30 dairy cows) were without bedding and elevated boxes; the original preronic system for excrement removal (entry of surface water) did not work. Excrements were removed using faecal cars, which caused problems during unfavourable climatic (spring and autumn) seasons. Both the disinfection and premises cleaning were irregular. As well, disinfestations and disinfection were performed irregularly, or almost not at all. The herd was supplemented with its own high-pregnant heifers reared by pasture rearing where mastitis occurred very often before, or after parturition. The hygienic programme of milking was not carried out correctly; the most frequent imperfections consisted in imperfect udder washing and drying, late dipping of the teat ends after milking. No control of the disinfectant solution concentrations was performed. There was also a shortage in disposable towels that were replaced by insufficient amount of cloth towels either for washing or drying of the udder. Clinical cases of mastitis were treated intra-mammary using antibiotics without the determination of the agent and preceding sensitivity determination. Bacteriological control of the treatment efficacy was not performed. A breeder ensured required number of somatic cells in the pool sample (400 000. l ml^{-1}) of milk according to the norm in such a way, that he selected dairy cows for milk supply to the dairy tank. Selection of dairy cows was carried out basing upon the results of NK test, the whole herd was examined in a week intervals. The dairy cows manifesting positive reaction to 2+, even if they milked only from each quarter of the udder separately and milk was discarded (the breeder performed this procedure also during our observation).

Accepted measures and therapy of dairy cows

The measures for removing the imperfections were accepted at the beginning of the observation. The breeder in accordance to valid norms cleaned and disinfected the housing premises, performed disinfection and disinfestations and accepted control measures for the activities for next period. He ensured control of a proper

function of the milking equipment by a service. A schedule for a subsequent control of function and procedures of daily, weekly, monthly cleaning and disinfection of the milking equipment was worked out. Milkers and livestock specialists were trained for a correctness of the individual steps of the hygienic programme at milking.

The incidence of mammary gland inflammations was observed within half a year in 3 month intervals (days 0, 92, and 184). At the complex examination of all lactating cows in the herd the udder of each cow was clinically investigated, and secretion of each lactating mammary gland was examined using the NK–test. From the udder of each dairy cow an individual sample of milk for bacteriological examination was collected under aseptic conditions.

After the initial complex examination of dairy cows (day 0) during 25 days 115 dairy cows were treated with the preparation Synulox LC susp. a. u. v. (Pfizer AH, Italy) and 6 dairy cows - by Tetra-delta susp. a. u. v. (Norbrook, Laboratories Ltd., Northern Ireland):

– 6 dairy cows with acute mastitis, 18 dairy cows with sub-acute mastitis, 10 dairy cows with chronic mastitis and 27 dairy cows with sub-clinical mastitis were transferred into two individual sections and their therapy started on day 5. After finishing the therapy, this group was milked as the first one. On day 2 following complex examination, 6 dairy cows with parenchymatous mastitis were excluded from the herd;
– 12 dairy cows with sub-clinical mastitis and 48 dairy cows with latent mastitis were transferred into two individual sections and their therapy was started on day 15 following complex examination. After finishing the therapy, this group was milked as the first one, i.e. before the sooner treated group of dairy cows.

From the beginning of the observation, every dairy cow entering the dry period was treated with antibiotic preparation after last milking and the teat ends were dipped into 50 % Jodanal M. The choice of the antibiotic preparation was based upon the results of the agent sensitivity to routinely used antibiotics for mastitis treatment determined at the complex examination of the herd.

After the second complex examination of dairy cows (day 92 of the observation) during 24 hours 26 dairy cows were treated with the preparation Synulox LC susp. a. u. v. (Pfizer AH, Italy), and 4 dairy cows with the preparation Tetra-delta susp. a. u. v. (Norbrook, Laboratories Ltd., Northern Ireland);

– 3 dairy cows with acute mastitis, 2 dairy cows with sub-acute mastitis, 11 dairy cows with chronic mastitis, and 16 dairy cows with sub-clinical mastitis were transferred into two individual sections and their therapy started on day 7 from the second complex examination. After finishing the therapy this group was milked as the first one;
– 4 dairy cows with chronic parenchymatous mastitis and 2 dairy cows with acute mastitis were culled out of the herd on day 2 after complex examination.

At the period between complex examinations of the herd a “fast” treatment of clinical cases of mastitis was done. The choice of the antibiotic preparation came from the results of the agent sensitivity to antibiotics, which were determined at the complex examination of the herd.

Intra-mammary preparations Synulox LC susp. a. u. v. (Pfizer AH, Italy) and Tetra-delta susp. a. u. v. (Norbrook, Laboratories Ltd., Northern Ireland) were used according to the instructions of the producer.

Therapeutic efficacy of the preparations used was determined on the basis of bacteriological examination of secretion of the mammary gland on days 7, 14, and 21 from the last administration of the preparation.

Examining procedures and methods

The history data on the health status of the dairy cow udders and correctness of the application of technological procedures were obtained regularly during the whole observation. A complex examination of the health status of the udders was carried out in 3 month intervals and included clinical examination of the udder, estimation of the first sprays of milk, milk examination by NK-test with subsequent collecting of individual milk samples (mixed quarters’ samples) for bacteriological examination, and subsequent cultivation and identification of pathogenic bacteria (IDF, 1981 and 1987). Isolation of bacteria was performed on the Columbia Blood Agar Bass (Oxoid, England) with 5 % addition of defibrinated ram blood, Staphylococcus medium No 110 (Oxoid, Basingstoke, Hants, England), Baird Parker agar (Oxoid, England), Edwards agar (Oxoid, England), Endo agar (Biomark, India). In the *Staphylococcus* sp. bacteria the coagulase test was carried out using Microbiology bactident coagulase test (Merck, KGaA Darmstadt, Germany).

Determination of the sensitivity to antibiotics was carried out by the diffuse disc method on Műeller-Hinton agar (NCCLS, 2002) using test discs with following amounts of effective substance: Amoxycillin-Clavulanat (20μg+10μg); Ampicillin (10 μg); Erythromycin (15 μg); Lincomycin (10 μg); Neomycin (30 μg); Novobiocin (30 μg); Oxacillin (5 μg); Penicillin (10 U); Streptomycin (10 μg); Cefalotin (30 μg), and Cefaperazone (75 μg).

RESULTS AND DISCUSSION

In table 1 both the representation of the bacterial agents of mastitis and number of infected dairy cows at individual examinations of the herd are presented.

At the beginning of the observation on day 0, of 174 dairy cows in the production cowshed 127 were infected. Thirteen dairy cows (7.47 %) were infected with the main contagious pathogenic bacteria. Fifty-nine dairy cows (33.91 %) manifested environmental pathogenic bacteria in the udder secretion. Secondary pathogenic bacteria were isolated from the udder secretion of 55 dairy cows (31.61%). During the next period 121 dairy cows were treated – 115 ones with the preparation Synulox LC susp. a. u. v. (tab. 3) and 6 ones with the preparation Tetra-delta susp. a. u. v. (one dairy cow with infection *Escherichia coli*, 2 infected with *Proteus* sp. bacteria, and 3 infected with *Bacillus* sp. bacteria, in all cases successfully). Six cows were culled out of the breeding (5 dairy cows at the first lactation infected parallelly with *Arcanobacterium pyogenes* and coagulase-negative staphylococci with parenchymatous mastitis with the opening communicating outwardly and one dairy cow at the first lactation with parenchymatous mastitis infected with *Arcanobacterium pyogenes* with healed wound on the udder). These dairy cows were replaced by highly pregnant heifers.

On day 92 of the observation 179 dairy cows underwent a complex examination. From the results of bacteriological examination of milk samples it followed that there were 36 (20.11 %) infected dairy cows in the herd. Thirty dairy cows were treated with the intra-mammary antibiotic preparations on the basis of determined sensitivity against antibiotics with subsequent bacteriologic control of the therapy – 26 dairy cows with the preparation Synulox LC susp. a. u. v. (tab. 4), and 4 ones with the preparation Tetra-delta susp. a. u. v. (3 dairy cows with *Escherichia coli* infection and l cow with *Bacillus* sp. infection, therapeutic intervention was in all cases successful). Six dairy cows were culled out of the herd (4 dairy cows at the first lactation with *Arcanobacterium pyogenes* infection and 2 ones with *Escherichia coli* infection). In this period, the greatest representation among the infections of the mammary glands of dairy cows had *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, and coagulase-negative staphylococci.

At the last complex examination of the herd on day 184 of the observation in 12 dairy cows (6.74 %) out of 178 ones the presence of bacterial agents of mastitis was recorded, whilst the environmental pathogenic bacteria were represented most (especially *Streptococcus uberis)* than secondary pathogenic bacteria and from the udder of one dairy cow *Staphylococcus aureus* was isolated.

During 6 months in the observed breeding we managed to reduce the incidence of mastitis from 72.99 % to 6.74 %. The applied anti-mastitis measures pronouncedly limited mastitis induced by *Arcanobacterium pyogenes.*

Table 1: Representation of bacterial agents of mastitis and number of infected dairy cows at individual examinations in the herd

Bacteriological finding	Period of observation					
	Day 0		Day 92		Day 184	
	n	%	n	%	n	%
Main pathogenic bacteria – infectious						
Staphylococccus aureus	0	0.00	0	0.00	1	0.56
Streptococcus agalactiae	13	7.47	1	0.56	0	0.0
Total	13	7.47	1	0.56	1	0.56
Environmental pathogenic bacteria						
Streptococcus uberis	48	27.59	6	3.35	4	2.25
S. uberis+CoNS	1	0.57	0	0.00	0	0.0
Streptococcus dysgalactiae	4	2.30	12	6.70	1	0.56
E. coli	1	0.58	5	2.79	1	0.56
Proteus sp.	2	1.15	0	0.0	1	0.56
Bacillus sp.	3	1.72	1	0.56	0	0.00
Total	59	33.91	24	13.40	7	3.93
Secondary pathogenic bacteria						
Koaguláza–negatívne stafylokoky	8	4.60	7	3,91	3	1.69
Arcanobacterium pyogenes	25	14.37	4	2.24	1	0.56
Arc. pyogenes+CoNS	22	12.64	0	0.00	0	0.00
Total	55	31.61	11	6.15	4	2.25
Infected dairy cows total	127	72.99	36	20.11	12	6.74
Non-infected total	47	27.01	143	79.89	166	93.26
Number of dairy cows in the herd	174		179		178	

CoNS = coagulase-negative staphylococci

In Table 2, there are results of clinical examination of the mammary gland and NK test. The data fully correspond to the results of bacteriological examination and the number of infected dairy cows in the herd observed. Pronounced reduction in the number of dairy cows with clinically apparent forms of mastitis, subclinical and latent mastitis testifies to a favourable effect of the anti-mastitis methods. This fact is also reflected by the results of NK test where after 3 months the number of positive dairy cows decreased by more than 2/3, and after 6 months by more than 5/6 compared to the original state.

In Table 3 the therapeutic efficacy of the intramammary preparation Synulox LC is presented at therapy of infected dairy cows after the first complex examination of the herd. The intra-mammary preparation showed the best therapeutic efficacy at the treatment of infection with *Streptococcus agalactiae* bacteria, *Streptococcus dysgalactiae* and mixed infection with *Streptococcus uberis* and coagulase-negative staphylococci, where it had 100.0 % therapeutic efficacy. Its least efficacy was at the infections caused by coagulase-negative staphylococci (therapeutic efficacy of 87.5 %). The overall therapeutic efficacy of the preparation (102/115 dairy cows, i.e. 88.71 %) against cured pathogenic bacteria can be evaluated as very good.

Table 2: Results of clinical examination of the mammary gland and NK-test in the breeding of dairy cows

Period of observation	Number of cows examined	Forms of mastitis					NK-test	
		Clinically apparent		Subcl.	Lat.	Abact.		
		Acute	Subac+Chron.					
	n	%	%	%	%	%	np	%
Day 0	174	6.9	16.1	22.4	27.6	1.7	130	74.4
Day 92	179	0.6	2.2	5.0	8.4	3.9	43	24.0
Day 184	178	0.0	0.56	3.4	2.8	1.7	21	11.8

n = number; np = number of positive; % = mastitis incidence in %; Subac.+Chron. = Sub-acute and Chronic mastitis total, Subcl. = Sub-clinical; Lat. = Latent; Abact. = A-bacterial

Table 3: Therapeutic efficacy of the intramammary preparation Synulox LC susp. a. u. v. (Pfizer AH, Italy) at therapy of infected dairy cows after the first complex examination of the herd

Pathogen	Number of dairy cows treated	Number of dairy cows cured	% cured dairy cows
Streptococcus agalactiae	13	13	100.00
Streptococcus uberis	48	45	93.75
Streptococcus uberis +CoNS	1	1	100.00
Streptococcus dysgalactiae	4	4	100.00
CoNS	8	7	87.50
Arcanobacterium pyogenes	24	23	95.80
Arcanobacterium pyogenes + CoNS	17	16	94.10
Total	115	102	88.71

CoNS = coagulase–negative staphylococci

Table 4: Therapeutic efficacy of the intramammary preparation Synulox LC susp. a. u. v. (Pfizer AH, Italy) at the therapy of infected dairy cows after the second complex examination of the herd

Pathogen	Number of dairy cows treated	Number of dairy cows cured	% cured dairy cows
Streptococcus agalactiae	1	1	100,00
Streptococcus uberis	6	6	100,00
Streptococcus dysgalactiae	12	11	91,67
CoNS	7	7	100,00
Total	26	25	96,15

CoNS = coagulase-negative staphylococci

Table 5: Results of sensitivity against antibiotics in *Streptococcus sp.* bacteria determined by the disc method

ATB – effective substance	S. uberis n = 52			S. dysgalactiae n = 14			S. agalactiae n = 14		
name	S (%)	IS (%)	R (%)	S (%)	IS (%)	R (%)	S (%)	IS (%)	R (%)
Amoxycillin/Clavulanat	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0
Ampicillin	100.0	0.0	0.0	85.7	14.3	0.0	100.0	0.0	0.0
Erythromycin	61.6	26.9	11.5	78.6	21.4	0.0	85.7	14.3	0.0
Lincomycin	80.8	17.3	1.9	71.5	21.4	7.1	100.0	0.0	0.0
Neomycin	61.6	26.9	11.5	85.8	7.1	7.1	57.1	28.6	14.3
Novobiocin	65.4	19.2	15.4	78.6	21.4	0	85.7	7.1	7.1
Oxacillin	67.3	21.2	11.5	78.6	14.3	7.1	100.0	0.0	0.0
Penicillin	73.1	25.0	1.9	64.3	21.4	14.3	100.0	0.0	0.0
Streptomycin	63.4	15.4	21.2	85.7	14.3	0.0	64.3	28.6	7.1
Cefalotin	86.5	7.7	5.8	100.0	0.0	0.0	-	-	-
Cefoperazone	94.2	5.8	0.0	100.0	0.0	0.0	-	-	-

n – number of strains; (S) - sensitive; (R) - resistant; (IS) - inter-border sensitivity

Table 6: Results of sensitivity against antibiotics in coagulase–negative staphylococci and *Arcanobacterium pyogenes* determined by the disc method

ATB – effective substance	Arcanobacterium pyogenes n = 47			CoNS n = 30		
názov	S (%)	IS (%)	R (%)	S (%)	IS (%)	R (%)
Amoxycillin/Clavulanat	93.6	6.4	0.0	90.0	6.7	3.3
Ampicillin	93.6	4.3	2.1	63.3	23.3	13.4
Erythromycin	59.6	34.0	6.4	66.7	23.3	10.0
Lincomycin	70.2	25.5	4.3	76.7	16.6	6.7
Neomycin	48.9	31.9	19.2	73.3	20.0	6.7
Novobiocin	59.6	25.5	14.9	76.7	20.0	3.3
Oxacillin	76.6	21.3	2.1	80.0	16.7	3.3
Penicillin	83.0	10.6	6.4	60.0	23.3	16.7
Streptomycin	80.9	12.7	6.4	70.0	16.7	13.3

n – number of strains; (S) - sensitive; (R) - resistant; (IS) - inter-border sensitivity

In Table 4 the therapeutic efficacy of the intra-mammary preparation Synulox LC is presented at the therapy of infected dairy cows after the second complex examination of the herd. The intra-mammary preparation showed the best therapeutic efficacy at the treatment of infection with bacteria *Streptococcus agalactiae*, *Streptococcus uberis* and coagulase-negative staphylococci, where 100.0 % therapeutic efficacy was reached. Very good therapeutic efficacy (91.67 %) was also obtained at the infections caused by *Streptococcus dysgalactiae*. The overall therapeutic efficacy - 96.15 % (25/26 dairy cows) reached in this case, was excellent.

Based upon the results of the sensitivity against antibiotics of the bacteria *Streptococcus* sp., presented in Table 5, it is apparent that *Streptococcus agalactiae* still persists its extreme sensitivity against classical penicillin, whilst it is equally sensitive against oxacillin, combination of amoxicillin with clavulanat, ampicillin and lincomycin. Surprising is the lower sensitivity against novobiocin, however, the sensitivity against neomycin and streptomycin could be expected regarding a wide range of minimal inhibitory concentrations in

this pathogen. Sensitivity of *Streptococcus dysgalactiae* is good except for higher occurrence of resistant strains against penicillin. Unfavourable state of sensitivity against antibiotics at *Streptococcus uberis* in given breeding is the result of uncontrolled using of antibiotic preparations for treatment of mastitis.

The results of sensitivity against antibiotics in the bacteria *Arcanobacterium pyogenes* presented in Table 6 revealed the weak sensitivity against neomycin and novobiocin. This pathogen also showed lower sensitivity against erythromycin and lincomycin. But taking into consideration only the number of sensitive strains against penicillin and streptomycin its sensitivity against these antibiotics can be considered as a lower. The results of sensitivity of coagulase-negative streptococci indicate a lower sensitivity against all antibiotics tested, except for the combination amoxicillin with clavulanat. As very low sensitivity it is necessary to consider the sensitivity against penicillin, ampicillin, and erythromycin, but also against streptomycin, neomycin, neomycin, novobiocin, and lincomycin, whilst sensitivity against oxacillin is not the best.

Reduction in the occurrence of dairy cows with intra-mammary infections of the udder in the herd by 66.25 % is higher when compared to the previous results (Vasil', 1999). A therapeutic efficacy of Synulox LC susp a. u. v., regarding the forms of mastitis cured and determined sensitivity of agents could be expected. Representation of etiological agents of environmental mastitis in dairy cows is almost the same as reported in the literature. Norwegian authors (Waage et al., 1999) during one year in 24 veterinary districts revealed an occurrence of clinical mastitis in 1040 heifers (with 1361 affected quarters). In infections following pathogenic bacteria were participated: *Staphylococcus aureus* (44.3%), *Streptococcus dysgalactiae* (18.2%), mixed infections *Staphylococcus aureus* and *Streptococcus dysgalactiae* (1.2%), coagulase–negative staphylococii (12.8%), *Arcanobacterium pyogenes* (3.5%), mixed infections *Arcanobacterium pyogens* with *Streptococcus dysgalactiae* (0.5 %) and *Staphylococcus aureus* (0.4 %), *Escherichia coli* (6.4 %). Of coagulase-negative staphylococci most represented were *Staphylococcus simulans* (53.7%), *Staphylococcus hyicus* (14.8%) and *Staphylococcus chromogenes* (14.8%). *Arcanobacterium pyogenes* bacteria were reported to be isolated almost four times more frequently before parturition than after heifer calving.

English authors (Hillerton and Berry, 2005) reported that the mastitis incidence in dairy cows induced by *Streptococcus* sp. bacteria locally and geographically varies, whilst in overall *Streptococcus uberis,* as a causal agent of clinical mastitis in dairy cows in the Great Britain participates about at 1/3 and in a lot of herds it is a dominant pathogen. As well, they point out that during 1964-2000 a pronounced change occurred in the representation of bacterial agents in the Great Britain. As an example, they introduce the herd of cows of the Institute for Animal Health, where during 36 years the incidence of clinical cases of mastitis induced by *Staphylococcus aureus* decreased from 43 % (in 1964) to 16 % (in 2000).

On the other hand, during that period the number of clinical mastitis in dairy cows induced by *Streptococcus uberis* increased from 20 % to 33 %, and number of cows with clinical mastitis induced by Gram-negative bacteria from 2,4 % up to 43 %. They state that during a year about 50 % of all lactating dairy cows in the Great Britain have infection of at least one quarter. At average about 25 % dairy cows in the herd are affected by clinical mastitis, whilst in the herd there are the mastitis incidence 40 cases per 100 cows.

The results of the testing of sensitivity of Str*eptococcus* sp. bacteria against antibiotics are almost the same as those reported by the authors from Germany (Trolidenier et al., 2000), when they tested 368 strains of *Streptococcus* sp. for the sensitivity against benzylpenicillin, ampicillin, oxacillin and cefotaxin. The strains of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* were almost all sensitive against these antibiotics (98–100.0 %), but from the strains of *Streptococcus uberis* only 83.2 % and 79.7 % were sensitive against oxacillin and benzylpenicillin, respectively.

Japanese authors (Yshimura et al., 2000) tested 42 bovine strains of *Arcanobacterium pyogenes*. All the strains were sensitive against benzylpenicillin, ampicillin, erythromycin, lincomycin, 7.1 % of strains were resistant to gentamicin, 52.1 % were not sensitive to DH-streptomycin. In comparison to above results, at our testing a high number of intermediary sensitive strains against erythromycin, lincomycin, neomycin, novobiocin, oxacallin, streptomycin, as well as classical penicillin were found.

The northern authors (Jousimies-Somer et al., 1996) reported about testing of 16 bacteria *Actinomyces pyogenes*, 8 bacteria *Streptococcus dysgalactiae*, 3 bacteria *Streptococcus uberis,* that were isolated from "summer" mastitis of dairy cows to the sensitivity against penicillin G, spiramycin, amoxicillin, combination of amoxicillin with clavulanat and ofloxacin, and each of them was sensitive to these antibiotics.

The incidence of mastitis induced by environmental agents of mastitis is a whole-world problem. Mastitis caused by *Streptococcus* sp., *Staphylococcus aureus*, *Arcanobacterium pyogenes*, *Escherichia coli* and *Klebsiela* sp. bacteria pronouncedly reduce milk production and decrease its quality (Wilson et al., 2004). In addition, we seriously have to focus our attention to mastitis induced by environmental agents regarding health

harmlessness of milk as a food, because it has a significant impact on the mortality of human population. From the point of view of national economics a prevention of this disease would be most effective. The basic principle of mastitis prevention consists in a reduction of the exposure of the teat ends of the mammary gland to the environmental pathogenic bacteria, and in stimulation of protective mechanisms of the udder (Hogan et al., 1989). The capability of environmental agents of mastitis to survive in the external environment outside the udder is not fully explained. However, infected quarters of the udder are most often the reservoir of infection for other dairy cows. New infections occur most often during a milking process (Tančin et al, 2006). Keeping cleanness of dairy cows, dry housing environment and application of all the measures, leading to minimization of the teat end contamination with pathogenic bacteria between milking, as well as during milking process, belong to the basic ways for prevention of infections from the external environment (Vasil', 2007).

CONCLUSION

In the herd with the mean number of 177 lactating dairy cows with the high incidence of mastitis (main agents of intra-mammary infection were *Streptococcus sp., Arcanobacterium pyogenes* and coagulase–negative staphylococci bacteria) the occurrence of dairy cows with intra-mammary infections of the udder was reduced from 72.99 % to 6.74 % during 6 months of application of preventive anti-mastitis measures and antibiotic intra-mammary therapy of bacteriologically positive dairy cows. The measures reduced the number of dairy cows with clinically apparent forms of mastitis, sub-clinic and latent mastitis. Effectiveness of the procedures are also reflected in the results of NK-test, when after 3 months the number of positive dairy cows decreased by more than 2/3 and after 6 months by more than 5/6 compared to the original state.

The incidence of mastitis induced by *Arcanobacterium pyogenes* was pronouncedly limited. The results of the observation show that an introduction of preventive inhibitory anti-mastitis methods at the high incidence of environmental mastitis requires a complex herd analysis and respecting of all factors influencing their origin. The focus of the measures has to reside in ensuring suitable housing of dairy cows, keeping the hygienic principles in breeding, application of preventive anti-mastitis methods (especially hygienic programme of milking and correct function of the milking equipment), but also early antimicrobial and anti-inflammatory therapy of clinical forms of mastitis. This allows us successfully oppose the incidence of this disease and ensure a production of health harmlessness safe milk.

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