JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

PREVENTIVE METHODS IN REDUCTION OF MASTITIS PATHOGENS IN DAIRY COWS

František Zigo[1], Juraj Elečko[1], Zuzana Farkašová[1], Martina Zigová[2], Milan Vasil'[1], Silvia Ondrašovičová[1], Kudělková Lenka[3]*

Address(es): DVM. František Zigo, Ph.D.
[1]University of Veterinary Medicine and Pharmacy, Department of Animal husbandry, Košice, Komenského 73, 041 81, Slovakia.
[2]University of Pavol Jozef Šafárik in Košice, Faculty of Medicine, Department of Pharmacology, Trieda SNP 1, 040 11 Košice, Slovakia.
[3]Department of Animal husbandry and zoohygiene, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic.

*Corresponding author: frantisek.zigo@uvlf.sk

doi: 10.15414/jmbfs.2019.9.1.121-126

ARTICLE INFO

Received 9. 11. 2018
Revised 4. 6. 2019
Accepted 4. 6. 2019
Published 1. 8. 2019

Regular article

OPEN ACCESS

ABSTRACT

Mammary gland tissue inflammation (mastitis) is the most frequent disease in dairy cattle in the world, and negatively influences the milk quality having consequences for the dairy processing industry. The aim of the study was analyze designed preventive and control methods focused to reduction of mastitis in herd of Slovak pied cattle in the east of Slovakia during two years of experiment. From 180 cows at quarterly intervals in the 3rd, 6th, 9th and 12th month was performed a complex examination of health udder including an assessment clinical signs of mammary gland, abnormal udder secretions, Californian Mastitis Test (CMT) with subsequent collecting of milk samples for bacteriological examination. In the first year during the first two complex examinations, treatment of mastitis caused by coagulase positive staphylococci (*S. aureus)*, coagulase negative staphylococci (*S. haemolyticus, S. warneri, S. epidermidis)* and *Str. agalactiae*, a reduction in prevalence from the original 41.3 % to 32.1 % was achieved. During the last two complex examinations in the first year the prevalence decreased to 25.2 % and then at the end to 21.1 %, respectively. The reduction of mastitis during the second year is characterized by a 22.1 %, 19.2 %, 12.2 % to 7.3 % mastitis, when the prevalence dropped by 5.5 %, respectively. Coagulase negative staphylococci and *Str. agalactiae* were the most numerous in each case during the second year and their occurrence subject to a proportional reduction. Proposed antimastitis methods and their implementation of continuous mastitis control system during two years, significantly reduced prevalence of mastitis by 34.0 % and influenced the occurrence of the most common pathogens of the mammary gland in monitored herd of dairy cows. Recorded reduction of mastitis in monitored dairy herd is an example of using available scientifically validated methods in a rationally compiled mastitis control program for the specific conditions of each dairy farm in the long period.

Keywords: Cows, Prevention, Mastitis, Machine milking, Bacterial pathogens

INTRODUCTION

Milk products from cows, sheep and goats are unique, especially in the field of rational nutrition of consumers. Many of milk products and specialties can be included among the functional foods for their nutritional value. The economic value of dairy cows is determined mainly by their milk yield and longevity, because milk is the main source of income on dairy farms. The main important factors affecting the quantity and quality of milk produced is the occurrence of production diseases, especially mastitis **(Vršková *et al*., 2015).**

Worldwide, mastitis (intramammary infection) is known as a multifactorial disease, and it is closely related to the production system and the environment that the cows are kept in (**Tančin *et al*., 2006).**

The most recent estimates from the National Mastitis Council (2001) suggest that mastitis affects one third of all dairy cows and will cost the dairy industry over 2 billion dollars annually in the United States in lost profits. The incidence of mastitis increases when defense mechanisms of the mammary gland are impaired. Dairy cattle are exposed to numerous genetic, physiological, and environmental factors associated with the host, pathogens that can compromise host immunity and increase the incidence of mastitis. Among the most infectious agents causing mastitis and reduction of milk yield belongs bacteria **(Pecka-Kiełb *et al*., 2016)**, viruses, fungi and algae **(Tančin *et* Uhrinčať, 2014).**

Bacteria are the major source of mastitis. For an intramammary infection (IMI) to occur it is necessary for the teat skin to be contaminated with pathogens, the pathogens to penetrate the teat duct and the infection to be established in the sinuses, ducts or tissues of the udder (Figure 1). With the inflammation follows an increase in the level of white blood cells or leukocytes, and this causes an increase in the somatic cell count (SCC) of the milk. The leukocytes are produced as a response to the injury or infection, and they are a crucial part in fighting the damage of tissue and infective agents **(Zadoks *et al*., 2001; Vasil' *et al*., 2016)**.

The prevalence of the IMI varies with the breed, age of the cows, milk yield and the stage of lactation. More than 50% of mastitis cases occurring during peripartal period or in the first two months after calving (**Huijps *et al*., 2008; Sharif *et al*., 2009; Zadoks *et al*., 2011**).

Based on the intensity and severity of clinical signs, mastitis is usually divided into subclinical and clinical disease **(Sztachańska *et al*., 2016).** In clinical mastitis (CM), signs range from mild to severe and can be systemic, local, or milk related, whereas in subclinical mastitis (SM), no signs are observed. During SM the udder and milk appears normal, but infection is still present. Due to the lack of symptoms, SCC can be used to indicate the prevalence of mastitis. In CM the clinical signs are clear. The most prominent symptoms of CM are swelling, heat, hardness, redness or pain of the udder. The milk of a cow with CM has a watery appearance, and flakes, clots or pus is often present.

Subclinical cases of mastitis are more common than clinical cases of mastitis. On average in the herd there are 15 - 40 undetected cases of SM for every CM in cows. There is an increase SCC what leads to reduced milk production to the tune of 60 to 140 liters per cow per year in subclinical mastitic animals **(Hameed *et al*., 2008; Sinha *et al*., 2014).**

Treatment of CM incurs costs that vary with the severity of the case and the response of the farmer. Determination of the cost of a case of CM can be complex given that losses can result not only from decreased production and discarded milk, but labor and medication costs, premium loss and penalties, culling and replacement costs, mortality and even impaired reproduction (Table 1). Unlike subclinical mastitis, the largest losses due to clinical disease are from discarded milk. Research regarding economic losses associated with mastitis differ greatly depending on the study and have been variously estimated at between $50 and $400 per case. As a rule of thumb, however, losses related to a single case of mastitis are typically put at somewhere between $100 and $200 **(Hillerton *et* Berry (2005).**

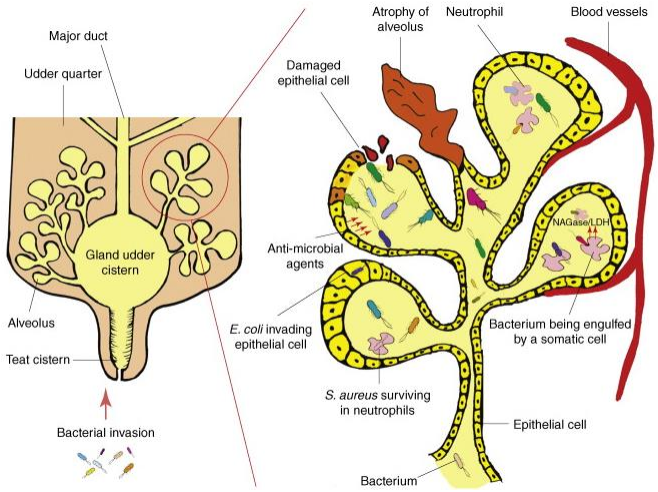

Figure 1 Environmental and contagious microorganisms invade the udder though the teat cistern. They then multiply within the alveolus where they are attacked by neutrophils (white blood cells) while damaging the milk-producing epithelial cells of the bovine udder
Source: **Viguier *et al.* (2009).**

Procedures across multiple versions of programs for prevention and control mastitis known as Mastitis Control Program, in principle, are designed to ensure a standard level of nutrition, hygiene of environment, and the proper functioning of the milking machine, targeted antibiotic treatment of inflammation during lactation, non-selective treatment of the udder at the beginning of drying-off, as well as the elimination of dairy cows with chronic mastitis from next breeding **(Pyörälä *et* Taponen 2009).** The diversity of the aetiology, the current sensitivity to antibiotics, rearing technology and breeding status, however, often requires the modification **(Bradley *et* Green, 2007).**
The aetiology and prevalence of the mastitis in a dairy herd of Slovak Pied cattle were analysed with the application of continued methods of control and prevention to reduction of IMI caused by pathogens bacteria during two years.

Table 1 Cost of an average case of clinical mastitis and bacterial pathogens in a dairy cow producing 7000 kg milk per lactation

Factor	Cost (£)	Bacterial pathogens	Positive identifications (%) from 100 clinical cases of IMI
Labour, 2 h at £6	12	**Coliforms**	43
Treatment, drugs and vet	3 - 11	***Streptococcus* spp.**	33 - 36
Discarded milk	26	*Streptococcus uberis*	30 - 33
Production loss (10%)	135	*Streptococcus dysgalactiae*	1 - 3
Reduced food intake	- 56 - 25	***Staphylococcus* spp.**	16 - 18
Fatality (1%)	3	*Staphylococcus aureus*	10 - 14
		CNS*	2 - 4
Total	**131**	***Trueperella pyogenes***	1 - 2

Legend: CNS* - coagulase negative staphylococci, IMI – intramammary infection
Sources: **Berry *et al.* (2004)** and **Hillerton *et* Berry (2005).**

MATERIAL AND METHODS

Animals and milking

The study was realized in herd of 180 Slovak pied dairy cattle (Zemplin) with standard zootechnic and zoohygienic conditions during two years. On the farm there are two brick stables where cows were kept on deep litter with free housing system. The zootechnicians office and the cloakroom were in the building, along with a milking parlour, which was followed by a covered waiting room. All cows were fed total mixed ration based on grass silage, maize silage, hay and concentrate according to international standards **(NRC, 2001)** to meet the nutritional requirements of a 600 kg cow, yielding 15 - 25 kg of milk/d and were allowed *ad libitum* access to water. Dirty litter was cleared and exchanged 2 times a week using a UNC mechanism.
The cows were milked twice a day in tandem milking parlor DeLaval 2x5 (Tumba, Sweden), with the first milking starting at 4.30 h in the morning and the second afternoon at 16.30 h. First, the wet toilet was performed with water to remove impurities from udder and teats. Subsequently, the udder was thoroughly wiped disposable paper wipes. The first milk from each quarter were hand-drawn into a dark-bottomed pot, and the milk was sensitively assessed. Milking and pulsation vacuum was set at 42 kPa. Pulsation ratio was 60:40 at a rate of 52 c/min and termination was automatically signalled when the milk flow dropped to 0.2 l/min. After milking process, the teats were disinfected in the form of teat-dipping. Milk was stored in refrigerating milk tanks at + 5 °C and removed daily around 11.30 hours.

Examination of health status and milk samples collection

A complex examination of all lactation cows were carried out quarterly in 3rd, 6th, 9th, and 12th of the month. After a veterinary history, cow udders, including sensory evaluation of the first milk, were examined. This was followed by an examination of all quarters using CMT (Indirect Diagnostic Test, Krause, Denmark) according to **Jackson *et* Cockcroft (2002)** (Table 2 and Figure 2). The CMT score interpretation in table 2 is expressed as the average percentage of individualy mastitis forms from all four investigations during first and second year. Mixed quarter samples of cow's milk (10 ml) were then collected by aseptic techniques in accordance with the guidelines of the **National Mastitis Council (2001)**. The cooled samples were immediately transported to the laboratory of University of Veterinary Medicine and Pharmacy in Kosice. Based on clinical signs, CMT score and bacteriological examination of milk samples, IMI were classified as latent, subclinical and clinical cases according to **Vasil *et al.* (2009)**.

Laboratory analysis

Bacteriological examinations and identification were performed according to generally accepted principles **Malinowski *et* Kłosowska (2002)**. Milk samples (10 µl) were inoculated on Petri plates with Columbia Blood Agar Base (Oxoid, UK) with 5% of defibrinated ram blood and incubated for 48 h at 37° C; the plates were examined after 24 and 48 h of incubation. A milk sample was classified as positive if at least two colony of *Staphylococcus aureus* or *Streptococcus agalactiae* was identified. For other bacteria, the presence of at least five to seven typical colonies was required for positive classification. Suspected colony were inoculated and cultured on selective nutrient soils such as Staphylococcus medium N°110 (Fig. 3), Baird-Parker agar, Brilliance^{TM} UTI Clarity Agar, Edwards Medium, Mac Conkey Agar (Oxoid, OXOID Ltd., Basingstoke, Hants, UK). Parameters such as colony size and appearance, pigment production and coagulase, catalase activity, hemolysis, Gram staining have also been taken into account in the determination of bacterial species. Colonies of *Staphylococcus* spp. were selected for a test for coagulase in a tube (Staphylo PK, Imuna Pharm, SR). Growth-confirmed colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were detailed identified biochemically using the STAPHYtest 24, STREPTOtest 24, resp. ENTEROtest 24 (Erba-Lachema, CZ) and identification by 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).

Table 2 Evaluation of milk samples and interpretation of CMT score

CMT score	SCC	Interpretation	Monitored herd	
			First year (%)	Second year (%)
N (negative)	0 - 200,000	Healthy quarter	67.6	82.2
T (trace)	200,000 - 400,000 (±50,000)	Latent mastitis*	3.9	2.3
1	400,000 - 650,000 (±150,000)	Subclinical mastitis*	14.8	7.9
2	850 000 - 1,200,000 (±200,000)	Subclinical mastitis Serious mastitis*	5.6	3.5
3	1,500,000 - 5,000,000 (±300,000)	Serious mastitis	5.3	2.5
4	Over 5,500,000	Serious mastitis	2.8	1.6

Legend: CMT - The Californian Mastitis Test, SCC- somatic cell count, Latent mastitis* - milk appears normal, but infection is still present in samples of raw milk without changing of SCC with negative CMT score, Subclinical mastitis* - no signs are observed, the udder and milk appears normal, but infection is still present with positive CMT score and increased SCC. Serious* or clinical mastitis – signs range from mild to severe with positive CMT score, bacteriological cultivation, high level of SCC, changing the consistency of the milk with the presence of flakes, clots or pus and reduction or loss of milk production with clinical signs.

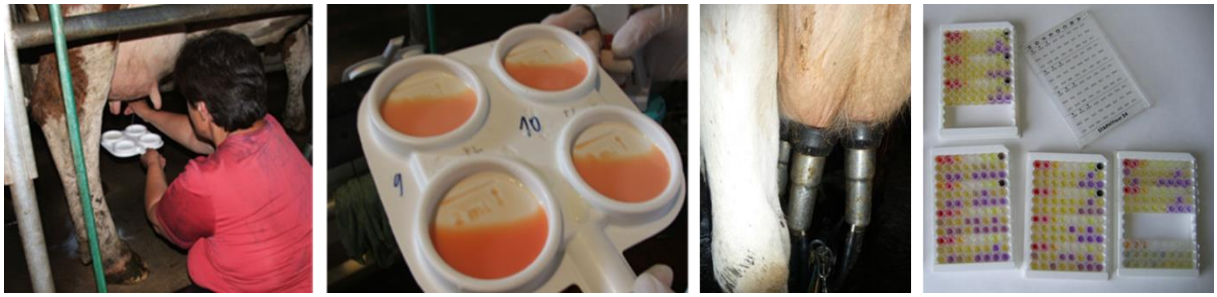

Figure 2 From left: Assessment of the first pre-milked samples with the evaluation of CMT score, machine milking and laboratory identification of bacteria *Staphylococcus* spp. using biochemical STAPHY-test

Suggested methods of damping mastitis during two years in monitored herd

Good routines for hygiene and treatment is the most important step for prevention of mastitis. From the anamnesis and analysis of the first examination of the herd of dairy cows, was designed the Mastitis Control Program which consist from:

a) renew the bedding materials frequently, preferable daily and do not keep cows in dirty paddocks

b) maintain good foot health

c) pre-milking hygiene - udder toilet: washing udder (have ready the udder cloths, basket preparation for dirty udder cloths), assessment of the first pre-milked samples into the vessel - cup with double bottom and control of milk consistency for presence of flakes, pre-dipping (disinfection of teats before milking with Valiant (ABS, CZ), drying of udder before milking

b) after milking - disinfection of all teats with IODERM 5000 (Hypered, CZ)

c) keeping the cows out of lying areas for 30 minutes after milking

d) cows with mastitis must be separated from healthy cows and individually milked as the last

e) all acute and subacute cases of mastitis treat according to actually sensitive to antibiotics process

f) monitoring milk quality and composition of the treated animals after inclusion in the milking

g) dairy cows with chronic mastitis or atrophy of secretory tissue in udder quarters after unsuccessful treatment must be rejected because they represent a constant reservoir for infection for the other cows in the herd.

h) unselective treatment of udder with antibiotics, on the start dry period

i) new dairy cows (predominantly gestative) can be integrated to herd after completely control of health status

j) keep the milking machine serviced

k) the control of right observance of designed methods.

Figure 3 From left: *S. aureus* and *S. warneri* cultured on blood agar base with 5% of defibrinated blood and Staphylococcal medium N ° 110

RESULTS AND DISCUSSION

A diagnosis of mastitis is based on clinical observations or direct or indirect measures of the inflammatory response to infection, whereas a diagnosis of an intramammary infection is based on identification of the infectious agent. SCC is a common diagnostic tests for the detection of SM, as well as the use of CMT. Culture and PCR can be useful in the diagnosis of an IMI. However, both have their advantages and disadvantages. Diagnosing the bacterial agent causing the intramammary infection can help to determine treatment and prevention strategies on the farm, which in turn can help to reduce incidence and prevalence **(Adkins *et* Middleton, 2018).**

The prevalence and aetiology of mastitis from mixed milk samples of 180 dairy cows during two years in eight examinations of experiment are described in Tables 3 and 4. In first year were 32.4 % cows positive of CMT reaction (Table 2) and from 29.6 % of cows` mixed milk samples were cultivated bacterial pathogens. During the first planned screening was prevalence of mastitis 41.3 % (55 positive cows` mixed milk samples) in the first year. The results of first examination (Table 3) are reflection of detected anamnesis of herd and its status of completely breeding activity. After reading the results of the first examination by a breeder, the herd began to apply measures such as: the creation of groups of dairy cows under production and stage of lactation, ensuring the implementation of appropriate disinfectants and devices for milking, treating during lactation and mammary gland treatments at the beginning of drying-off. In particular the treatment of clinical mastitis already after three months to reach the reduction of the prevalence from 41.3 % to 32.1 %, and preferably treated dairy cows with the findings of *S. aureus*, CNS, *Str. agalactiae* and *Trueperella pyogenes* from

positive milk samples. In the first year, after the third examination, the prevalence decreased (25.2 %), and then at the end of the first year of the experiment with the standardization of hygiene in milking the value of 21.1 % was recorded. In second year were 17.8 % cows were positive of CMT reaction (Table 2) and from 15.3 % of cows` mixed milk samples were cultivated bacterial pathogens. Effect of applied controlling methods during the second year (Table 4), reduced the incidence of the apparent mastitis 22.8 %, 19.2 %, 12.2 % and 7.3 %, respectively, while there was a decline in the prevalence of 5.5 % in the course, or for the entire experiment of 34.0 %.

Table 3 Prevalence and etiology of mastitis from cow's milk samples in four examinations during the first year of monitoring

First year (sequentially accepted methods of prevention and reduction of mastitis)								
Examination/Milk samples	**I./133**		**II./137**		**III./143**		**IV./147**	
Isolated bacteria	n	%	n	%	n	%	n	%
***Staphylococcus* spp.**	**41**	**30.9**	**20**	**14.5**	**16**	**11.2**	**16**	**10.9**
S. aureus	*9*	*6.8*	*4*	*2.9*	*1*	*0.7*		
S. haemolyticus	*4*	*3.0*	*7*	*5.1*	*3*	*2.1*	*6*	*40.1*
S. warneri	*7*	*5.3*	*1*	*0.7*	*3*	*2.1*	*2*	*1.4*
S. epidermidis	*2*	*1.5*	*1*	*0.7*			*3*	*2.0*
S. chromogenes			*3*	*2.2*	*2*	*1.4*		
S. sciuri	*11*	*8.3*						
S. schleiferi					*6*	*4.2*	*5*	*3.4*
S. xylosus			*4*	*2.9*	*1*	*0.7*		
S. lentus	*8*	*6.0*						
Other pathogens bacteria								
Streptococcus agalactiae	7	5.3	5	3.6	10	7.0	8	5.4
Trueperella pyogenes	2	1.5	7	5.1	4	2.8	3	1.4
mixed infection*	5	3.7	12	8.8	6	4.2	4	2.7
Prevalence of mastitis	***55***	***41.3***	***44***	***32.1***	***36***	***25.2***	***31***	***21.1***

Legend: n – number of samples with positive cultivation of bacterial pathogens, mixed infection* - mixed infection caused two or more bacteria (*Enterococcus spp.*, *E. coli* and *Bacillus spp*.)

Different types of IMI are caused by different bacterial species. Some bacteria prefer environmental niches, others are contagious, and many are opportunistic. According to the authors **Sharif *et al.* (2009)**, the most contagious mastitis pathogens causing IMI are *Staphylococcus aureus*, *Str. agalactiae* and *Streptococcus uberis*. The main reservoirs of contagious pathogens are bovine tonsils, rumen, rectal, genital regions and mammary gland itself. Intramammary infections caused by contagious pathogens can vary from subclinical to clinical mastitis. Subclinical infection often goes unnoticed. Long lasting subclinical infection can sometimes progress to a clinical mastitis with drastic changes in milk (clotting, hemorrhage) and in the udder (pain, swelling), as well as systemic signs (fever, loss of appetite). *S. aureus* with *Str. agalactiae* were observed to be the most common cause of CM in our study.

Their transmission in the herd is thought to be strictly contagious, i.e. from cow to cow, due to insufficient hygiene in the milking parlor, allowing multiple animals to come into contact with equipment, hands or towels that are contaminated by milk from an infected cow. Frequency of contagious pathogens among CM cases is greater. The use of dry cow therapy, post milking teat disinfectants and effective pre-milking hygiene are effective control procedures for most contagious mastitis pathogens **(Sori *et al*., 2005)**.

The treatment of mastitis by antimicrobials produces residues in milk, which is an important aspect to consider. CM should in general be treated with narrow-spectrum antimicrobial, and first choice for treating infections caused by streptococci and penicillin-susceptible staphylococci are β-lactam antimicrobials. It is recommended to treat both systemically as well as intramammary for at least three days (**Pyörälä, 2009**).

Among environmental pathogens, the most common bacteria are *Str. dysgalactiae*, coliforms such as *E. coli* and *Klebsiella*. Coagulase negative staphylococci are also the environmental bacterial pathogens of increasing importance in udder infections. Their are normal inhabitants of the skin and teat canal and may be frequently isolated from milk samples. In recent decades, CNS have become among the most common mastitis-causing agents in well-managed dairy farms in many countries **(Orwin *et al*., 2001; Taponen *et al*., 2006; Sameer *et al*., 2018)**. *S. chromogenes, S. simulans, S. xylosus, S. haemolyticus, S. warneri* and *S. epidermidis* are the most common mastitis-causing CNS species (**Pyörälä *et* Taponen, 2009**).

Particularly *S. chromogenes, S. epidermidis, S. haemoyticus* and *S. warneri* may have pathological significance of IMI with increase in mean milk SCC **(Zigo *et al*., 2017)**. Bacteria of *Staphylococcus* spp. (Tab. 3 and 4) were the most numerous and each time of experiment they were subject to a proportional reduction. For the duration of the experiment in addition to the coagulase-positive *S. aureus* were isolated other 10 types of CNS. From these *S. haemolyticus* and *S. warneri* which have been isolated on a regular basis, especially in the first year, along with other species such as S. *chromogenes* and *S. epidermidis* caused clinical and subclinical mastitis most frequently. Sporadic findings of *S. schleiferi, S. xylosus, S. lentus, S. sciuri* and *S. capitis* were isolated usually in cases of latent mastitis which are characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency. The last examination on the end of second year was characterized by finding only 4.0 % of the staphylococci (*S. haemolyticus, S. chromogenes* and *S. epidermidis).*

Mastitis caused by CNS usually displays relatively mild clinical signs, and these bacteria can therefore affect milk quality for a long period before being noticed. In contrast, *Streptococcus uberis* is a widely distributed environmental pathogen causing more severe signs. The environmental pathogens are more difficult to eradicate due to their ubiquitous presence and they remain a major challenge to the dairy industry (**Hogan et Smith, 2012**). They can be controlled by reducing exposure and by increasing immune resistance of the cow by post milking teat dipping with a germicidal and treatment of all quarters with antibiotics during drying off **(Fox *et* Gay, 1993)**. In the studies conducted by **Monday *et* Bohach (1999)** and **Thorber *et al*. (2009)** were CNS the most prevalent pathogens causing SM in dairy cows and ewes. Although less pathogenic than *S. aureus*, CNS can also produce persistent subclinical or clinical mastitis. After infection of CNS is significantly increased SCC, CMT, cause CM as well as producing thermostable enterotoxins. Nevertheless, despite the accepted role of these bacteria as major mastitis causing pathogens in cows and ewes. The pathogenicity of the different CNS species varies widely.

The most frequently isolates from CNS species in subclinical and chronical mastitis are *S. epidermidis*, *S. caprae, S. simulans*, *S. chromogenes* and *S. xylosus* **(Bergdoll *et* Lee Wong, 2006)**.

Thorberg and colleagues **(2009)** confirmed and demonstrated one or two types of *S. epidermidis* in two monitored herds of dairy cows. The dominant types of *S. epidermidis* from milk were also isolated from skin of the people who were responsible for milking cows because isolation of *S. epidermidis* from human skin is more common than isolation from bovine skin. The authors conclude that humans who are daily in contact with animals are probably the main source of infection for cows.

Other types of bacteria in our study were represented by *Str. agalactiae, Trueperella pyogenes, E coli, Enterococcus* spp. and *Bacillus* spp. during monitored period The importance of using the results of diagnostics is manifested even in control of owns cows, and its inclusion into the herd rearing. Designed by continuous year lasting mastitis control system significantly reduced the incidence and the prevalence of the most common pathogens affected mammary gland. Given the variety of factors causing the IMI milk production and economic prosperity will depend on the expertise of the farmers to quickly implement preventive anti-mastitis methods to their own dairy production.

The study done by **Hillerton *et* Berry (2005)** confirmed that the implementation of the mastitis control program does not completely exclude IMI from the herd, but it is effective in keeping the incidences at a low level. However, advances in detection systems have not brought efficient cow-side methods to achieve this

better care. Usually annually, in a resting or dry period, all dairy animals must spend anywhere from 6 to 10 weeks prior to caving in a non-lactating phase. The cow remains especially susceptible to the contraction of IMI soon after carving, and the secession of milking or "drying off" period.

Table 4 Prevalence and etiology of mastitis from cow's milk samples in four herd examinations during the second year of monitoring

Second year (application of mastitis control program)								
Examination/Milk samples	**V./149**		**VI./151**		**VII./156**		**VIII./151**	
Isolated bacteria	n	%	n	%	n	%	n	%
Staphylococcus spp.	**14**	**9.5**	**10**	**6.6**	**13**	**8.3**	**6**	**4.0**
S. aureus	*1*	*0.7*			*1*	*0.6*		
S. chromogenes	*3*	*2.0*			*3*	*1.9*	*2*	*1.3*
S. epidermidis	*4*	*2.7*					*1*	*0.7*
S. haemolyticus			*1*	*0.7*	*5*	*3.2*	*3*	*2.0*
S. warneri	*5*	*3.4*	*3*	*2.0*				
S. capitis					*4*	*2.6*		
S. sciuri			*2*	*1.3*				
S. schleiferi			*4*	*2.6*				
S. xylosus	*1*	*0.7*						
Other pathogens bacteria								
Streptococcus agalactiae	6	4.0	9	6.0	1	0.6	3	2.0
Trueperella pyogenes	4	2.7	3	2.0	2	1.3	1	0.7
mixed infection*	10	6.7	7	4.6	3	1.9	1	0.7
Prevalence of mastitis	***34***	***22.8***	***29***	***19.2***	***19***	***12.2***	***11***	***7.3***

Legend: n – number of samples with positive cultivation of bacterial pathogens, mixed infection* - mixed infection caused two or more bacteria (*Enterococcus spp.*, *E. coli* and *Bacillus spp*.)

CONCLUSION

A unique and continuous progress of the reduction of mastitis (in the first of a partial, or total in the second year) by implementation of the mastitis control program is the result of the interplay of effects applied to the methods of prevention that provide protection against the emergence of new infections and disease control methods (treatment and rejection), which drastically reduce the duration of the infection. Constant observance of the hygiene practices in milking, treatment of dairy cows by making effective therapies in cows with clinical mastitis, and disposal of cows with the chronic form has been reduced the incidence of clinical mastitis in the herd to minimum. Rich knowledge of systematic research into the problem of reducing the presence of mastitis at home and in the world confirm the need to take into account the polyetiological and multifactorial character of IMI in the everyday practice of farmers. It is therefore necessary to implement new knowledge and technological processes in dairy farming in order to achieve the highest quality of produced milk.

Acknowledgments: This work was supported by grants APVV no. SK-PL-18-0088, VEGA no. 1-0529-19 (Slovak) and project Cultural heritage of small homelands no. PPI-APM-2018-1-00010-U-001 (Poland).

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