

# IDENTIFICATION OF MASTITIS ETIOLOGIC AGENTS IN LITHUANIAN CATTLE HERDS

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## ABSTRACT

The aim of this study was to analyse the cow mastitis situation during 1998–2007. Somatic cell count and bacteriological investigations were the main methods used in this study. According to the data obtained, there was noticed a frequent increase of somatic cells count in the milk of examined cows that demonstrates the outspread of mastitis in herds. There were totally tested 58,908 cows affected with mastitis during the analysed period. As a reason of mastitis in 57.6 % cases was defined *S. aureus*, in 15.3 % – *S. agalactiae*, in 6.36 % – *Enterobacter spp.*, in 0.36 % – *P. aeruginosa* and in 0.46 % – *Corynebacterium spp.* 61.7 % of all mastitis samples were examined during the housing period, and only 17.6% – during December-February.

**KEY WORDS:** cows, mastitis, *S. aureus*, SCC.

## INTRODUCTION

Mastitis is the mammary gland inflammation, which is usually caused by microorganisms. Microorganisms infect the milk, may produce toxins, that cause animal health to worse, change the constitution and characteristics of milk. Therefore the raw milk becomes not qualitative, inappropriate for industrial usage. *S. aureus* and *Streptococcus spp.* are the most common pathogens of mastitis [9]. The frequency of the pathogens is different in different breeding regions of the world. Mastitis is spread among 30-40 % of dairy cows in the United Kingdom and Sweden. In Finland, the reason of mastitis in 19.42 % was defined *S. aureus*, in 3.8 % – coagulase negative staphylococci, in 4.91 % – *S. uberis*, [10]. In Denmark, the cause of mastitis in 90% was staphylococci, streptococci, coliforms [11]. Identification of the pathogens, causing mastitis is very important, because only the correct diagnosis can eliminate the pathogens of mastitis in the herd. The unified methodology of staphylococci and streptococci identification was introduced in Lithuania in 1996. Therefore it is possible to identify the pathogen of mastitis and explicit the results.

The aim of the study was to analyse the cow mastitis situation in the period of 1998-2007 and to determine the most common causative agents spread in Lithuania.

## MATERIALS AND METHODS

Examinations were carried out using a retrospective research method during 1998-2007 in Lithuania. The data were collected at National Veterinary Laboratory (NVL) and government enterprise State Laboratory “Pieno tyrimai“. Milk of mastitis affected cows for laboratory testing was collected from all regions of Lithuania in different size cow herds during various seasons in 1998-2007. The pathogens of mastitis were identified in compliance with a standardised methodology (“Laboratory and field handbook on bovine mastitis“, National mastitis council, 1987) with some new knowledge of some species. Somatic cell count in the milk analyse during different years were carried out on SL “Pieno tyrimai” (accredited laboratory in compliance with the standard LST EN ISO/IEC 17025 requirements) examined dairy farm. Milk samples were analysed by fluorooptoelectronic method using device *Somaskope*. The precision of the quality of identification methods was provided according to

the decision of the European Council 91/180/EEB that regulates raw and thermal processed milk analyse methods.

Statistical analysis was performed by 'Epi Info' (1996; Centers for Disease Control & Prevention (CDC), U. S. A., Version 6.04). Arithmetical means (X), standard deviation (SD), vary coefficients (Cv) and set minimal and maximal meanings were calculated. The Student multiple comparison method was applied to determine the significance criterion for the difference among the groups (p). The difference was considered statistically significant when the "p" mean value was less than 0.05.

## RESULTS AND DISCUSSION

Somatic cells count (SCC) as a measure of quality of the milk and health of udder was investigated. 47.5 % of milking cows are controlled in Lithuania (Annual Report on Milk Recording), therefore, data analysed form the controlled population reflects the health of the cows in the country. According to SL "Pieno tyrimai" data, SCC increased in the controlled herds from  $464.09 \pm 45.71$  to  $617 \pm 53.87$ . Increased statistical significance ( $p < 0.05$ ) was recorded since year 2000 (Table 1). The data of SCC in the milk was compared to the number of mastitis samples (Table 2) investigated in laboratories. Results showed that SCC conversely correlated with the examined number of the mastitis samples ( $r = -0.66$ ).

During the period of ten years 25.976 cows with the signs of mastitis were examined in NVL, with average of 6.352 cows per annum (Table 3).

61.7 % of all samples were investigated during housing period (October – April), as usually cows were affected with mastitis on cowshed period rather on pasturage period (Table 2). Most of mastitis examinations were made from April till June, as at the end of winter season the immunity of the cows is weak and mastitis number increases when the pasture starts and intensive milk production begins. Most of the bacteriological examinations were made from the April till June (the end of the cowshed period and the beginning of the pasture period) and October - November, though according to data presented by SL "Pieno tyrimai", there were no difference in SC count (Table 2) in different months ( $p > 0.05$ ).

The analysis of the bacteriological examinations of the milk samples showed (Table 3) that the most common etiological agent of mastitis was *S. aureus* (57.6 %) and much more less – *S. agalactiae* (15.3%), *Enterobacter* spp. – (6.36 %), *P. aeruginosa* and *Corynebacterium* spp (0.36 % and 0.46 %, respectively). The remainder (16.6 %) consist of - *S. dysgalactiae*, *E. faecalis*, *E. faecium*, *S. hyicus*, *S. bovis*, *E. coli*, and other bacteria (Table 3). In different years the number of mastitis caused by *S. aureus* varied from 32.8 % to 73.4 % and this is significantly more ( $p < 0.05$ ) than total number of all other pathogens.

The announced SCC data shows critical udder's health condition. Inverse correlation reliance between SCC and bacteriologically confirmed mastitis shows that mastitis examinations are too expensive for the milk producers. Small milk farms are predominant in the country (Table 1) and control of clinical mastitis is too expensive. However a quite number of the bacteriological examinations (Tables 1 and 3) were performed during investigations. Since 1999, a new milk collection and quality investigation system has been introduced in Lithuania. The payment for the milk is based on the analysis results. It is individual, low-cost SCC examination method for every controlled cow. According to EU regulations, after reduction of the highest line in the geometrical average counting methodology, cows which milk contain high SCC and it reduction wasn't economically profitable, were eliminated from the herds. This influenced decreasing of cow number in the herds, so amount of bacteriological examination decrease too (Tables 1 and 3).

Table 1

**The variation of cows number, productivity, herds constitution and changes of mastitis morbidity in 1998-2007**

Rates	Years of the examination									
	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Cows numbers, (thousands)*	620,0	540,9	494,3	487,9	491,8	467,4	478,8	428,75	437,15	395,1
Milk quantity for lactation (kg)*	4125	4250	4447	4863	5015	5031	5231	5395	5592	5863
SCC in the bulk milk of controlled cows, thousands/ml	475,00 ±97,00	464,09 ±45,71	484,54 ±38,24	614,54 ±52,37	527,27 ±*57,50	590,00 ±37,95	514.54 ±49.92	617,0±5 3,87	506,2±5 0,23	581,6±5, 16
Herds %, having 1-5 cows*	87.0	75.7	74.9	73.0	65.2	61.2	62.2	54.2	45.0	19.0
The cows with mastitis investigated by NVL	10,215	13,369	4,271	4,035	3,675	4,685	4,875	5,658	4,237	3,888
<i>S. aureus</i> % in mastitis samples	84.9	73.5	68.2	64.0	48.7	64.5	57.6	65.9	41.3	39.1
<i>S. agalactiae</i> % in the masitis samples	15.0	16.8	16.9	23.7	18.0	21.8	19.3	12.9	18.2	17.7

P.S. \* according to Annual Report on Milk Recording

However, number of somatic cells in the milk of the controlled herd's cows remained high in the later years. Therefore, it can be assumed that a high number of subclinical mastitis cases exist in the herds in Lithuania. The situation is similar in other countries where data shows that 80-97 % of mastitis is subclinical [3].

Table 2

**SCC and bacteriologically examined mastitis quantity according to months**

Months	SCC, in thousands	Bacteriological quantity	Bacteriologically, %
I	504.44±127.682	176.92±20.315	2.5
II	480.55±138.122	445.85±39.264	6.3
III	455.55±146.787	445.85±79.452	6.3
IV	506.11±86.160	983.70±135.860	13.9
V	500.55±71.695	799.70±132.524	11.3
VI	474.44±78.598	714.77±103.762	10.1
VII	*	445.85±51.388	6.3
VIII	507.22±123.316	445.85±48.127	6.3
IX	485.00±112.360	261.89±45.218	3.7
X	464.44±88.049	891.70±125.164	12.6
XI	517.77±48.676	799.70±113.240	11.3
XII	563.33±54.772	622.77±97.989	8.8

\* In July the mean of SCC was extrapolated

Table 3

**NVL investigated pathogens from the mastitis milk samples**

Investigation years	Identified pathogens						
	Total	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>Entero-bacter</i> spp.	<i>P. aerugi-nosa</i>	<i>C. bovis</i>	Other
1998	4105	2440	432	332	3	1	897
1999	5569	4093	938	324	20	4	190
2000	1685	1174	326	147	4	2	32
2001	1803	1154	427	163	0	14	45
2002	1705	891	331	149	22	44	268
2003	2115	1365	462	124	10	0	154
2004	1994	1333	345	59	2	40	215
2005	2518	1661	325	88	1	6	441
2006	2289	946	417	128	28	44	726
2007	2193	858	390	267	34	10	634
Total	25976	14969	3976	1653	96	121	2876
Mean	2597,6	1663,2	441,7	183,6	10,6	13,4	319,5
X	1257,19	1027,65	193,10	99,91	11,96	16,81	287,86
SD	397,561	342,550	64,368	33,305	3,989	5,605	95,954

*S. aureus* is stated as the main and stable detected mastitis agent in Lithuania. In many countries where monitoring of *S. aureus* is performed in herds, the role of this pathogen is reduced during the years from 17.7% to 9.7% [8]. However, our research results do not show similar tendency in Lithuanian cows' herds [1, 5, 6].

*S. agalactiae* was detected as mastitis pathogen rarely. The frequency of this pathogen is similar to Latvian data, where *S. agalactiae* causes reported in approximately 15 % of mastitis [7]. Considering the data obtained in this study it should be stated that the significance of *S. agalactiae* is not decreasing among all pathogens of milk mastitis during the past years. Meanwhile, Makovec and Ruegg (2003) during the investigation of mastitis indicated that mastitis caused by *S. agalactiae* reduced from 8.1 to 3.0% while cases caused by other streptococcus increased from 11.6 to 20.1% [8].

Researches from Finland announced that *Corynebacterium bovis* becomes more frequent source of increasing SCC [5]. However, data in Lithuania shows that yeast resulted mastitis is only in 2.61% of all examined samples [1].

## CONCLUSIONS

1. Bovine mastitis is a serious problem of stockbreeding in Lithuania.
2. *S. aureus* and *S. agalactiae* predominate as etiological agents of bovine mastitis and there is no tendency to decrease the cases of mastitis causing by *S. aureus* in the country.

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