INFLUENCE OF MASTITIS AGENTS ON MILK TRAITS OF COWS

Eglė Čereškienė, Vida Juozaitienė, Arūnas Juozaitis, Janina Černauskienė, Judita Žymantienė, Jonė Kantautaitė

Veterinary Academy, Lithuanian University of Health Sciences
Tilžės St. 18, LT - 47181 Kaunas, Lithuania

Corresponding author: Eglė Čereškienė
e–mail: eglecr@gmail.com

Abstract. The present study was conducted to determine the presence of major mastitis agents in dairy cows herds in Lithuania and to investigate their influence on milk traits of cows. The research was carried out in the herds (n=85) of the dairy cows (n=4323), at the State Laboratory for Milk Control "Pieno tyrimai", at the Centre of State Rural Business Development and Information and at the Laboratory of Establishment of Animal Breeding Value and Selection of Lithuanian University of Health Sciences. The most frequent mastitis pathogens in herds of Lithuanian cows were mixed microflora agents (23.8 %) and *Staphylococcus aureus* (15.3%). The increse class of milk SCC has influenced the increase of frequency of *Staphylococcus aureus* and *Streptococcus agalactiae* (P<0.0001). Pathogens of mastitis cause many changes for milk composition of infected cows (P = 0.007-0.0001) and depend on invasion of pathogens to the tissues of mammary gland.

Keywords: cows, milk, udder, mastitis, pathogen

Introduction

Mastitis is an important production disease which causes huge economic losses to dairy industry in terms of production loss, milk loss due to disposal after treatment, treatment loss, man power loss as well as premature culling (Sharma, 2012).

According to their epidemiology, mastitis pathogens can be divided into contagious and environmental (Cervinkova et al., 2013). *Streptococcus agalactiae, Staphylococcus aureus subsp.aureus* and *Mycoplasma spp*. are considered as typical contagious pathogens; typical environmental pathogens are called environmental streptococci (streptococci other than *S. agalactiae* such as *Streptococcus uberis*; enterococci), Enterobacteria and coagulase-negative staphylococci (CNS).

S. uberis, Escherichia coli, Klebsiella spp., *Pseudomonas aeruginosa* and pyogenic bacteria are mainly considered as causative agents of clinical mastitis*; S. agalactiae*, CNS and *Enterococcus spp*. are associated with subclinical mastitis (Bradley 2002; Awale et al. 2012). *S. aureus* has been designated as a causative agent of both clinical and subclinical (Gruet et al. 2001; Awale et al. 2012) mastitis.

Mastitis influences the total milk output and modifies milk composition and technological usability. For cows, the somatic cell count (SCC) is a useful predictor of subclinical mastitis, and therefore, it is an important component of milk in terms of quality, hygiene, and mastitis control (Harmon, 1994).

Worldwide, farmers have achieved tremendous success in reducing the incidence of contagious mastitis by adopting the traditional methods of mastitis control. The greatest impact of these control measures has been on infections caused by the contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactia*. But this success has not been demonstrated for clinical mastitis caused by other agents. Organisms such as coagulase negative Staphylococci, environmental Streptococci, Mycoplasma spp and Serratia spp were isolated from dairy herds that had low somatic cell counts (Janus, 2009).

The present study was conducted to determine the presence of major mastitis agents in dairy cows herds in Lithuania and to investigate their influence on milk traits of cows.

Materials and methods

The research was carried out in the herds (n=85) of the dairy cows (n=4323), at the State Laboratory for Milk Control "Pieno tyrimai", at the Centre of State Rural Business Development and Information and at the Laboratory of Establishment of Animal Breeding Value and Selection of Lithuanian University of Health Sciences.

The research of milk composition, somatic cell count in milk and microbiological testing of milk samples from cows for diagnose of mastitis was performed at the State enterprise „Pieno Tyrimai" and covered the period from 2014 to 2016. The amount of milk fat, proteins and lactose was determined using the device "Lactoscope 550" ("Delta Instrumensts", Holland). "Lactoscope 550" measures the absorption of the specific wavelength of medial infrared rays of each component (LST ISO 9622:2000). The somatic cell count in milk was determined using the device "Somascope MK2", which functions by the principle of stream citometry (LST EN ISO 13366:1999-03). State enterprise "Pieno tyrimai" operates under quality management system conforming to the requirements of International Standard ISO/IEC 17025:2005 to ensure the accuracy of milk composition and quality tests.

Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990).

Cows were divided according to milk SCC (with SCC up to 200*10^3/ml - group I, 200-400*10^3/ml – group II and more than 400*10^3/ml – group III).

Statistical characteristics of the samples (arithmetic mean - M, standard error - SE), one way ANOVA and $\chi 2$ test were calculated using statistical software SPSS (version 15, SPSS Inc., Chicago, IL).

Results

Determination of causative agent is important for mastitis treatment and prevention for dairy cows. Figure 1 summarizes the prevalence of individual cow milk samples that were positive for the monitored microorganisms.

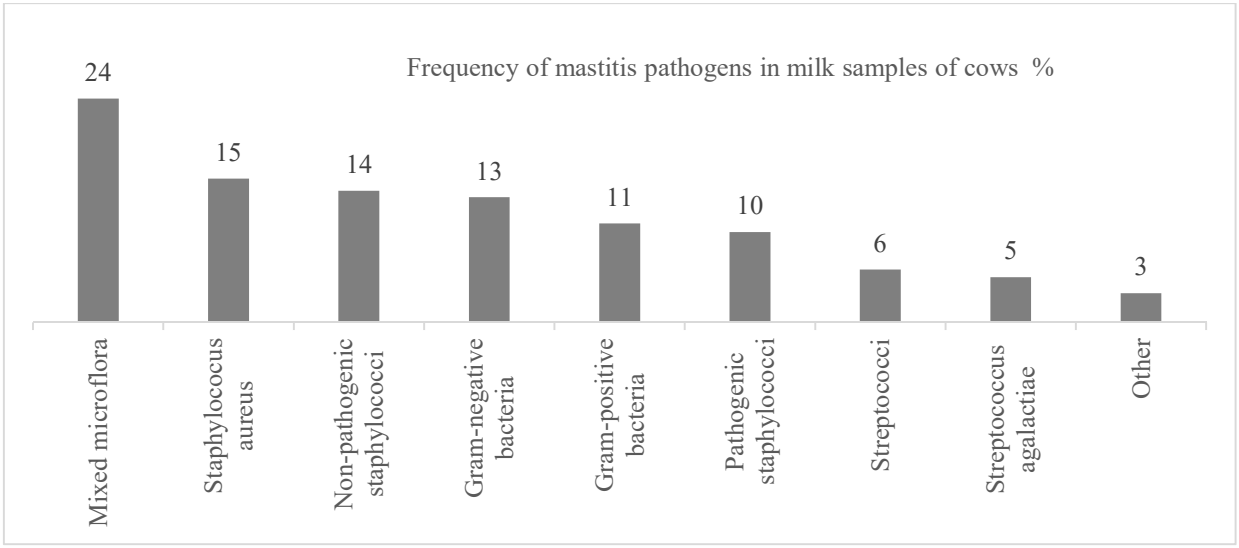

Figure 1. **Frequency of mastitis pathogens**

The most frequent mastitis pathogens in Lithuanian cow herd were mixed microflora agents (23.8 %). *S. Aureus* (15.3%) and *S. Agalactiae* (4.8 %) a contagious pathogens are usually spread through milking equipment, so it is very important to observe the milking hygiene. Frequency of other gram positive and negative bacteria - 23.8 %, non-pathogenic and pathogenic staphylococci – 23.6% was determined.

The average milk fat of the all investigated cows was 4.37±0.015%, protein – 3.47± 0.008%, lactose – 4.25± 0.007%.

Somatic cell count is an important tool for monitoring mastitis and general mammary gland health. The average somatic cell count in milk of cows was 709.41±21.182*10^3/ml. Cows with SCC in milk up to 200*10^3/ml amounted to 46.63 %, 200-400*10^3/ml – 18.32% and more than 400*10^3/ml – 35.05 %.

The investigation revealed that milk SCC statistically significantly negatively correlated with milk lactose ($r = -0.302$, $P < 0.01$), positive – with milk protein ($r = 0.200$, $P < 0.01$).

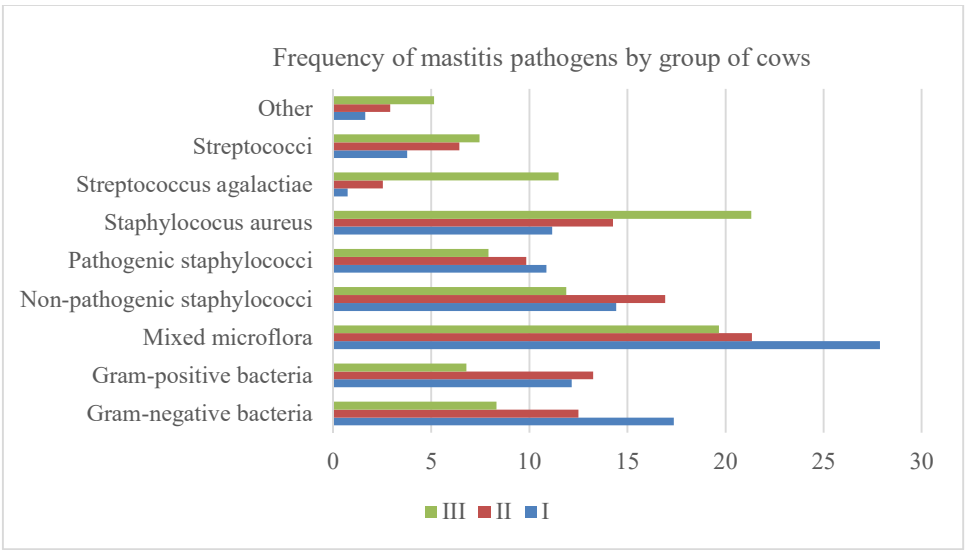

Figure 2. **Frequency of mastitis pathogens according to milk SCC class**

The analysis (Figure 2) shows that the increase of milk SCC class was associated with the incidence of mastitis pathogens (P<0.0001) and mostly influenced the frequency of *Staphylococcus aureus* and *Streptococcus agalactiae* (10% increase), Gram-negative bacteria (9% decrease) and mixed microflora (8% decrease).

ANOVA test demonstrated that mastitis pathogens were statistically significant associated with investigated milk traits of cows (P = 0.007-0.0001). The results are also described in the Table 1.

Table 1. **Milk traits of cows depending on the mastitis pathogens**

Mastitis pathogens	Statistic	Fat %	Protein %	Lactose %	Somatic cell count*10^3/ml
Mixed microflora	M	4.40	3.49	4.29	552.83
	SE	0.033	0.016	0.014	37.92
Staphylococus aureus	M	4.42	3.45	4.17	923.96
	SE	0.04	0.021	0.018	57.271
Non-pathogenic staphylococi	M	4.35	3.45	4.26	599.61
	SE	0.042	0.022	0.019	51.881
Gram-negative bacteria	M	4.29	3.41	4.33	466.11
	SE	0.038	0.022	0.019	43.404
Gram-positive bacteria	M	4.34	3.43	4.24	395.14
	SE	0.045	0.025	0.022	37.369
Pathogenic staphylococi	M	4.35	3.48	4.25	537.48
	SE	0.051	0.027	0.021	55.131
Streptococci	M	4.42	3.53	4.19	1022.43
	SE	0.06	0.038	0.032	110.864
Streptococcus agalactiae	M	4.31	3.55	4.10	2289.17
	SE	0.065	0.035	0.038	175.541
Influence of mastitis pathogens (ANOVA test)	P	0.007	0.000	0.000	0.000

According to mastitis pathogens milk fat content ranged from 4.29% (Gram-negative bacteria) to 4.42% (*Staphylococus aureus*), milk protein - from 3.43% (Gram-positive bacteria) – to 3.55% (*Streptococcus agalactiae*), milk lactose – from 4.10% (*Streptococcus agalactiae*) to 4.33% (Gram-negative bacteria), milk somatic cell count - from 395.14 *10^3/ml (Gram-positive bacteria) to 2289.17 *10^3/ml (*Streptococcus agalactiae*).

Discussion

Mastitis is the inflammation of the mammary gland and udder tissue, a major endemic disease of dairy cattle; it usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical or thermal injury to the cow's udder. Mastitis, as one of the most costly disease in the dairy industry, is the result of the interactions between a combination of microbiological factors, host responses in the udder, and management practices (Halasa et. al, 2007).

Over 135 different microorganisms (bacterial, algal or fungal) were isolated as bovine IMI (intramammary infection), but the majority of infections are caused by staphylococci, streptococci, and gram-negative bacteria (Watts, 1988).

The most prevalent mastitis pathogens in Lithuanian cow herd were mixed microflora agents and *S. aureus* (39.1%). The results of bacteriological findings are summarized in Figure 1.

Kalmus et al. (2011) and Abera et al (2013) reported that *S. aureus* was found to be the most prevalent organism isolated from milk samples.

Cervinkova et al. (2013) reported that coagulase-negative staphylococci clearly predominated (53.5% positive samples) followed by streptococci and enterococci (both occurring in 16.1% samples); among streptococci, so-called mastitis streptococci (S. *uberis, S. dysgalactiae* and *S. agalactiae*) prevailed (11.7% positive samples); yeasts (mainly *Candida spp*.) were found in 8.2% samples. One of the major mastitis pathogens, *Staphylococcus aureus*, was isolated from 9.0% of samples.

The most frequently isolated pathogen in Argentina (Dieser et al., 2014) was coagulase-negative staphylococci (CNS) (52.1%), followed by *Staphylococcus aureus* (21.3%), *Corynebacterium spp*. (5.2%), *Streptococcus agalactiae* (4.4%) and *Streptococcus dysgalactiae* (4.4%).

Milk composition is considered as an important factor for the dairy farmers to maintain raw milk quality, dairy industries to produce better quality dairy products and consumers to maintain nutritional quality and safety (Malek dos Reis et al., 2013).

In the study pathogens of mastitis cause many changes in milk composition (P = 0.007-0.0001). The lowest milk protein content had milk from infected animals with gram-positive bacteria, milk fat – with gram-negative bacteria.

Patbandha et al. (2015) determined that among several milk components, milk protein and lactose percent showed a significant difference (P<0.05) between milk samples from normal and inflamed quarters (during the early stage of mammary gland inflammation milk protein percent remained significantly high (P<0.05), later with an increase in the

degree of severity of inflammation it did not show any difference; milk lactose percent decreased gradually with an increase in the degree of severity of inflammation).

Lactose is synthesized in the gland cells of the udder from glucose and galactose during inflammatory reduced secretory activity at that mammary cells due to destruction of the epithelial cells by the leukocytes (Coulon et al., 2002).

Mastitis had significant influence on lactose content but there was a tendency for a decrease in this component with increase milk SCC.

The researchers (Bansal et al., 2005) found that lactose content was higher in healthy quarters. In clinical mastitis cases somatic cell counts are generally two to three times higher than uninfected quarters (Janus, 2009).

The highest SCC was observed in milk samples that were infected with *Staphylococus aureus* (923.96±57.271*10^3/ml), Streptococci (1022.43±110.864*10^3 ml) and *Streptococcus agalactiae* (2289.17 *10^3/ml).

Idriss et al. (2013) found that high frequency of mastitis pathogenic in high SCC >400*10^3/ml group of samples depending on the year were Coagulase Negative Staphylococci (CNS), Staph. aureus, yeasts, E. Coli, Bacillus sp. and Pseudomonas aerugenosa.

Conclusions

The most frequent mastitis pathogens in dairy cattle herd of Lithuania were mixed microflora agents and *Staphylococcus aureus* (39.1%). Pathogens of mastitis cause many changes for milk composition of infected cows and depend on invasion of pathogens to the tissues of mammary gland. Milk protein content was the lowest in milk from infected animals with gram-positive bacteria, milk fat – with gram-negative bacteria. Negative relationship between lactose content and milk SCC was observed. The increase of milk SCC class has influenced the increase of frequency of *Staphylococcus aureus* and *Streptococcus agalactiae* (P<0.0001).

References

1. Abera M., Habte T., Aragaw K., Asmare K., Sheferaw D. Major causes of mastitis and associated risk factors in smallholder dairy farms in and around Hawassa, Southern Ethiopia. Trop Anim Health Prod. 2012. Vol. 44. P. 1175-1179.

2. Awale M. M., Dudhatra G. B., Avinash K. Chauhan B. N., Kamani D. R., Modi C. M., Patel H. B., O'Kennedy R. Bovine mastitis: a threat to economy. Open Access Scientific. 2012. Reports 1. P. 295.

3. Bansal B. K., Hamann J., Grabowski N., Singh K. B. Variation in the composition of selected milk fraction samples from healthy and mastitis quarters, and its significance for mastitis diagnosis. J Dairy Res. 2005. Vol. 72. P. 144-152.

4. Bradley A. J. Bovine mastitis: an evolving disease. Veterinary Journal. 2002. Vol. 164. P. 116-128.

5. Cervinkova D., Vlkova H., Borodacova I., Makovcova J., Babak V., LorencovaA., VrtkovaI., MarosevicD., Jaglic Z. Prevalence of mastitis pathogens in milk from clinically healthy cows. Veterinarni Medicina. 2013. Vol.58 (11). P. 567-575.

6. Coulon J.B., Gasqui P., Barnouin J., Ollier A., Pradel P., Pomiès D. Effect of mastitis and related -germ on milk yield and composition during naturally-occurring udder infections indairy cows. Anim Res. 2002. Vol. 51. P. 383-393.

7. Dieser S. A., Vissio C., Lasagno M. C., Bogni C. I., Larriestra A. J., Odierno L. M. Prevalence of Pathogens Causing Subclinical Mastitis in Argentinean Dairy Herds. Pak Vet J., 2014. Vol. 34(1). P. 124-126.

8. Gruet P., Maincent P., Berthelot X., Kaltsatos V. Bovine mastitis and intramammary drug delivery: review and perspectives. Advanced Drug Delivery Reviews 2001. Vol.50. P. 245-259.

9. Halasa T., Huijps K., Osteras O., Hogeveen H. Economic effects of bovine mastitis and mastitis management [a review]. Vet Q. 2007. Vol. 29. P. 18-31.

10. Harmon R. J., Eberhart R. J., Jasper D. E. , Langlois B. E. , Wilson M. S. Bacteriological procedures for diagnosis of bovine udder infection. Arlington: National Mastitis Council, 1990. 34 p.

11. Harmon R. J. Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci. 1994. Vol. 7. P. 2103-2112.

12. Idris S. I., Tančin V., Foltys V., Kirchnerová K., Tančinová D., Vršková M. Relationship between mastitis causative pathogens and somatic cell counts in milk of dairy cows. Potravinarstvo. 2013. Vol. 7. No. 1. P. 207-212

13. Janus A. Emerging mastitis pathogens. Veterinary World. 2009. Vol. 2(1). P.38-39.

14. Kalmus P., Aasmäe B., Kärssin A., Orroand T., Kask K., Udderpathogens and their resistance to antimicrobialagents in dairy cows in Estonia. Acta Vet Scand. 2011. Vol. 53. P. 4.

15. Malek dos Reis C.B, Barreiro J.R, Mestieri L., Porcionato M.A., dos Santos M.V. Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. BMC Vet. Res. 2013. Vol. 9. P.67.

16. Patbandha T. K., Marandi S., Ravikala K., Pathak R., Maharana B. R., Murthy K. S. Association of milk components with intra-mammary inflammation in Jaffrabadi buffaloes. Vet World. 2015. Vol. 8(8). P.989-993.

17. Sharma N., Rho G.J., Hong Y.H, Kang T.Y, Lee H.K, Hur T.Y., Jeong D.K. Bovine mastitis: An Asian perspective. Asian J. Anim. Vet. Adv. 2012. Vol. 7(6). P. 454-476.

18. Watts J. Etiological agents of bovine mastitis. Vet Microbiol. 1988. Vol. 16. P. 41-66.

Received 15 June 2016
Accepted 01 July 2016