

The incidence of subclinical and clinical mastitis in dairy cows, their etiology and impact on reproductive indicators

Zuzana Lacková¹, František Zigo^{1*}, Anna Prnová¹, Ibrahim F. Rehan^{1,2}, Petra Timkovičová Lacková¹, Tomáš Mihok¹ Gokben Ozbey³

¹Department of Nutrition and Animal Husbandry, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

²Department of Biotechnology and Nutrigenomics, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Postepu 36A, 05-552 Jastrzebiec, Poland

³Department of Medical Services and Techniques, Vocational School of Health Services, Firat University, TR-23119 Elazig, Turkey

***For Correspondence**

Assoc. prof. DVM. FRANTIŠEK ZIGO, PhD.

Department of Nutrition and Animal Husbandry,
University of Veterinary Medicine and Pharmacy in
Košice, Komenského 73, Košice, Slovakia

frantisek.zigo@uvlf.sk

Abstract:

The aim of this study was to evaluate the occurrence and etiology of mastitis and its impact on the reproductive performance of dairy cows. A total of 112 dairy cows were monitored 10 days after calving, all with a history of increased somatic cell counts and a positive result in the California Mastitis Test (CMT). Clinical examination of the mammary gland was performed, followed by CMT testing and microbiological analysis of milk samples. Positive findings were recorded in 50 cows (43%), of which 25 showed subclinical mastitis and 25 exhibited the clinical form of the disease. Microbiological analysis revealed that the most frequently isolated bacteria were *Staphylococcus aureus*, coagulase-negative staphylococci (*S. xylosus* and *S. chromogenes*), *Aerococcus viridans*, and *Escherichia coli*. When compared with the reference values for reproductive parameters, dairy cows with clinical mastitis showed impaired reproductive performance, indicating a negative impact of mastitis on fertility and overall reproductive efficiency.

Keywords: mastitis, dairy cows, intercalation period, insemination interval, insemination index, service period



I. Introduction

The economic value of dairy cows is determined primarily by their milk yield and the number of lactations during their production period. Production diseases, especially mastitis, are among the important factors affecting the quantity and quality of milk produced [1]. Several types of microorganisms are involved in the development of infectious mastitis. Up to 90% of cases are bacterial infections, in a smaller number of cases infections by other organisms, such as viruses, fungi, yeasts, parasites and others. The number of types of bacterial causative agents of mastitis in dairy cows, reported in the literature, is large and apparently not yet complete. According to Sharif et al. [2], the main contagious bacterial pathogens causing mastitis include *S. aureus*, *Streptococcus agalactiae* and *Streptococcus disagalactiae*.

The most common environmental pathogens are *Streptococcus uberis*, *E. coli* and *Klebsiella*. Other environmental pathogens commonly found on the skin of the mammary gland include coagulase-negative staphylococci (CNS), especially *Staphylococcus chromogenes*, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus haemolyticus*, *Staphylococcus warneri* and *Staphylococcus epidermidis* [3]. Mastitis caused by these pathogens is often mild, but their long-term effects result in significant economic losses due to reduced milk yield or exceeding the established limit of the number of somatic cells count (SCC) and the total number of microorganisms. It is very important that all udder pathogens enter the milk and are excreted by it, but not always and in the same quantities. This depends on many circumstances, e.g. on the intensity of the disease, its form and duration, the resistance of the macroorganism, etc. [4].

Reproductive parameters in dairy cows are a key factor for milk producers and impaired reproduction is one of the causes of reduced production efficiency in the dairy industry. The key indicator of fertility in a dairy herd is fertility. The overall reproductive performance of the herd can be monitored from various aspects and a reproductive disorder can be considered if the parameters that the breeder has set with regard to breed, productivity and nutrition are not achieved. Reproductive indicators are used for a simple and quick assessment of the results of herd fertility management. The most important indicators of cow fertility include: age at first calving, service period, length of pregnancy, intercalation period [1].

The aim of the work was to evaluate the incidence and etiology of mastitis and their impact on the reproductive indicators of dairy cows.

II. Material and Methods

The investigated farm specializes in milk production and is located in the Trenčín region. The farm breeds 472 cattle (mostly Holstein type), of which 191 are dairy cows. We included in the study dairy cows that were 10 days after calving with a history of intramammary infection in the previous lactation. These were mainly dairy cows that had a positive CMT (indirect diagnostic test, Krause, Denmark) and an increased SCC value in the last three months before drying off. After the initial history, the dairy cows were clinically examined.

Milk from each quarter of the udder was subjected to sensory examination. The CMT was performed on all quarters of the milked cows. Subsequently, 12 ml of milk was aseptically collected from all quarters for laboratory analysis of bacterial pathogens. The samples were cooled to 4 °C and immediately transported to the laboratory and analyzed the next day. Bacteriological examination was performed according to the methodology of Malinowski and Kłossowska [4] with identification of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp. using the STAPHY-test, STREPTO-test or ENTERO-test and identified using the TNW Pro 7.0 software (Erba-Lachema, CZ) [5].

The following parameters such as insemination interval, insemination index, intercalation period, service period and lactation length were obtained from the databases of the given herd.

III. Results and Discussion

Table 1 shows the results of the evaluation of milk samples by CMT and microbiological examination. Out of the total number of 448 quarter milk samples assessed by CMT, 67 were positive. Out of 112 mixed samples of milk from dairy cows, 50 were positive microbiological findings, 25 positive findings in the subclinical form of

mastitis and 25 in the clinical form (Figure 1). In the subclinical form of mastitis, the following pathogens were identified: *Aerococcus viridans*, *Staphylococcus xylosus*, *Staphylococcus aureus*, *Staphylococcus chromogenes* and *Staphylococcus warneri*. In the clinical form, *Staphylococcus aureus*, *Staphylococcus chromogenes*, *Aerococcus viridans*, *Staphylococcus xylosus* and *Escherichia coli*.

Several authors have noted in their studies that *Staphylococcus* spp. are among the most common causes of intramammary infections in ruminants (*Staphylococcus aureus* in clinical forms and CNS in subclinical forms). The most common cause of CNS is *Staphylococcus epidermidis*. No less important bacterial pathogens are *Corynebacterium* spp., *Enterococcus* spp. and *Micrococcus* spp. [6, 7]. Finnish authors report that up to 50% of isolated pathogenic bacteria are CNS [8]. The second most frequently isolated pathogens are, according to Tenhagen et al. [9], *Streptococcus* spp. (12.6%). This conclusion correlates with the results of the identification of isolates (n = 1631) obtained from dairy farms in France [10], with *Streptococcus dysgalactiae* being the most prevalent agent. According to De Vliegher et al. [11], *Streptococcus uberis* is the most common causative agent of bovine environmental mastitis.

Table 1. Overview of CMT- test and microbiological examination results

Order of collection	No.	CMT result		Mikrobiological examination	
		Negative	Positive	Negative	Positive
1	56	190 (85%)	34 (15%)	21 (38%)	35 (62%)
2	26	95 (91%)	9 (9%)	15 (58%)	11 (42%)
3	30	96 (80%)	24 (20%)	26 (87%)	4 (13%)
Summary	112	381 (85%)	67 (15%)	62 (55%)	50 (45%)

Note: CMT - California Mastitis Test, No. – number of samples.

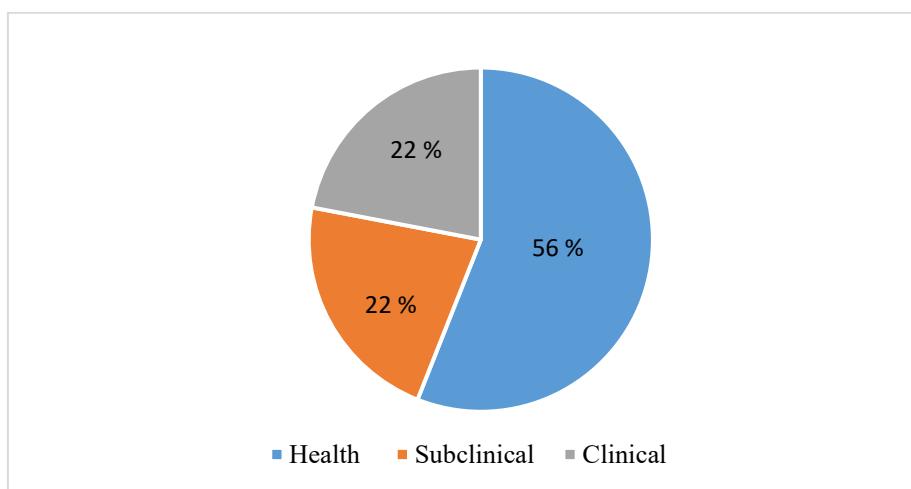


Figure 1. Incidence of subclinical and clinical mastitis on the farm

The results of the evaluation of dairy cow breeding based on reproductive parameters and the results of microbiological examination of samples are presented in Table 2. Of the monitored reproductive indicators, 26 dairy cows (23%) had unsatisfactory values for the length of the insemination interval, 46 dairy cows (41%) for the value of the insemination index, 13 dairy cows (12%) for the length of the service period, 12 dairy cows (11%) for the length of the intercalation period and 26 dairy cows (23%) for the lactation period. In dairy cows with unsatisfactory values of the reproductive parameter, the occurrence of SCM (subclinical mastitis) and CM (clinical mastitis) in varying numbers was recorded. Of the 26 dairy cows with unsatisfactory lengths of the insemination

interval, SCM (8%) and CM (15%) were recorded in 2 dairy cows. With unsatisfactory values of the insemination index, we recorded the occurrence of 7 SCM (15%) and 6 CM (13%), during the service period 4 SCM (31%) and 3 CM (23%), during the intermediate period 1 SCM (8%) and 1 CM (8%). The length of lactation was affected in dairy cows with 4 SCM (15%) and 7 CM (27%).

Fertility is an important factor for the profitability of breeding breeds with a focus on dairy, but also combined productivity. The analysis of reproduction indicators is quite complex, because their phenotypic variability is very large due to the large number of factors affecting fertility. The assessment of the length of the intercalation period is a selection criterion for fertility. The length of the intercalation period is determined by: insemination interval, service period and length of gestation. The intercalation period is closely related to the average lactation day in the herd, which has a great influence on milk production and also determines the rate of herd renewal, i.e. how many calves are born per year. As the intercalation period increases, the number of dairy cows removed from the herd due to reproductive problems increases. Hradecká et al. [12] found that when evaluating factors on reproduction indicators in cows, the year and month of calving, herd, age at first calving and breed have a statistically significant effect. Bujko and Rybanská [13] report that when evaluating production and reproductive indicators in the population of Slovak piebald cows, the herd, year and month of calving have a significant effect. Kumar et al. [14] and Dolecheck et al. [15] pointed out that mastitis during the breeding season has a negative effect on reproductive ability. Cows with mastitis have delayed estrus, reduced pregnancy and an increased risk of abortion. The systemic immune response and endocrine changes associated with mastitis can also disrupt follicular development, oocyte function and ovulation ability, further impairing fertility [16]. Mastitis causes a lengthening of the estrus interval and a reduction in the luteal phase in cows, which disrupts pregnancy maintenance and affects embryonic development [17].

Table 2. Evaluation of dairy cow breeding based on reproductive parameters and microbiological examination of milk samples

Parameter	Reproductive parameter value			Dairy cows with unsatisfactory reproductive parameter value	
	S [pcs/%)	U [pcs/%)	SCM [pcs/%)	CM [pcs/%)	Negative [pcs/%)
Insemination interval (55-80 days)	86 (77%)	26 (23%)	2 (8%)	4 (15%)	20 (77%)
Insemination index (1.2-2)	66 (59%)	46 (41%)	7 (15%)	6 (13%)	33 (72%)
Service period (60 -110 days)	99 (88%)	13 (12%)	4 (31%)	3 (23%)	6 (46%)
Interval period (365-400 days)	46 (41%)	12 (11%)	1 (8%)	1 (8%)	10 (84%)
Lactation period (240-305 days)	86 (77%)	26 (23%)	4 (15%)	7 (27%)	15 (58%)

Note: S – satisfactory, U – unsatisfactory, SCM – subclinical mastitis, CM – clinical mastitis.

IV. Conclusion

In mixed milk samples, *Staphylococcus aureus*, coagulase-negative staphylococci (*Staphylococcus xylosus* and *Staphylococcus chromogenes*), *Aerococcus viridans* and *Escherichia coli* were detected in both forms of mastitis. Compared to the optimal values of reproductive indicators, the results showed unsatisfactory reproductive values, especially in dairy cows with clinical mastitis.

V. Acknowledgements

This study was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0457: Non-antibiotic approaches to control mastitis of cows with an increase in the hygiene of dairy farms conditions and project VEGA no. 1-0162-23: Reduction in antibiotic use in dairy mastitis control programs.

References

- [1] M. Vršková, V. Tancín, K. Kirchnerová, P. Sláma, Evaluation of daily milk production in tsigai ewes by somatic cell count. *Potravinárstvo*, 9, 2015, 206-210, doi:10.5219/439
- [2] A. Sharif, M. Umer, G. Muhammad, Mastitis control in dairy production. *Journal of Agriculture & Social Sciences*, 5, 2009, 102-105.
- [3] S. Pyörälä, S. Taponen, Coagulase-negative staphylococci - Emerging mastitis pathogens. *Veterinary Microbiology*, 134 (2), 2009, 3-8, <https://doi.org/10.1016/j.vetmic.2008.09.015>
- [4] A. Malinowski, A. Kłossowska, Diagnostics of intramammary infections. National Veterinary Research Institute, Puławy, Poland, 2002.
- [5] F. Zigo, M. Vasiľ, J. Elečko, M. Zigová, Z. Farkašová, Mastitis pathogens and their resistance against antimicrobial agents in herds of dairy cows situated in marginal parts of Slovakia. *Potravinárstvo*, 12(1), 2018, 285-291, <https://doi.org/10.5219/905>.
- [6] D. Bergonier, R. Crémoux, R. Rupp, G. Lagriffoul, X. Erthelot, Mastitis of dairy small ruminants. *Veterinary Research*, 34, 2003, 689-716, <https://doi.org/10.1051/vetres:2003030>.
- [7] X. Berthelot, G. Lagriffoul, D. Concorde, F. Bariellet, D. Bergonier, Physiological and pathological thresholds of somatic cell counts in ewe milk. *Small Ruminant Research*, 62, 2006, 27-31, <https://doi.org/10.1016/j.smallrumres.2005.07.047>
- [8] A. Pitkälä, M. Haveri, S. Pyörälä, V. Myllys, T. Honkanen-Buzalski, Bovine mastitis in Finland 2001: prevalence, distribution of bacteria, and antimicrobial resistance. *Journal of Dairy Science*, 87, 2004, 2433-2441, doi: 10.3168/jds.S0022-0302(04)73366-4.
- [9] B.A. Tenhagen, I. Hansen, A. Reinecke, W. Heuwieser, Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *Journal of Dairy Science*, 76, 2009, 179-187, <https://doi.org/10.1017/S0022029908003786>
- [10] M.A. Botrel, M. Haenni, E. Mornignat, P. Sulpice, J.Y. Madec, D. Calavas, Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne Pathogens and Disease*, 7, 2010, 479-487. DOI: 10.1089/fpd.2009.0425
- [11] S. De Vliegher, L. Fox, S. Piepers, S. McDougall, H. Barkema, Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. *Journal of Dairy Science*, 95, 2012, 1025-1040, <https://doi.org/10.3168/jds.2010-4074>
- [12] E. Hradecká, V. Rehout, J. Citek, Evaluation of reproductive parameters in the Czech population of dairy cows. In: *Animal Science Papers and Reports*, 22(2), 2004, 45-48. RIV/60076658:12220/04:00005920
- [13] J. Bujko, M. Rybanská, Factors effecting the milk production in population of Slovak spotted breed. In: Book of XXI. Genetic days 2004, Wroclaw, Poland, 1.-3. Sept. 2004.
- [14] N. Kumar, A. Manimaran, A. Kumaresan, S. Jeyakumar, L. Sreela, P. Mooventhalan, M. Sivaram, Mastitis effects on reproductive performance in dairy cattle: a review. *Tropical Animal Health and Production*, 49, 2017, 663-673, doi: 10.1007/s11250-017-1253-4.
- [15] K.A. Dolecheck, A. García-Guerra, L.E. Moraes, Quantifying the effects of mastitis on the reproductive performance of dairy cows: a meta-analysis, *Journal of Dairy Science*, 102(9), 2019, 8454-8477, <https://doi.org/10.3168/jds.2018-15127>
- [16] I. Boujenane, J. El Aimani, K. By, Effects of clinical mastitis on reproductive and milk performance of Holstein cows in Morocco. *Tropical Animal Health and Production*, 47(1), 2015, 207-211, doi: 10.1007/s11250-014-0711-5.
- [17] I.N.F. Edelhoff, M.H.C. Pereira, J.J. Bromfield, J.L.M. Vasconcelos, J.E.P. Santos, Inflammatory diseases in dairy cows: risk factors and associations with pregnancy after embryo transfer, *Journal of Dairy Science*, 103, 2020, 11970-11987, doi: 10.3168/jds.2020-19070.