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Original Research Article

MICROBIOLOGICAL MONITORING OF MASTITIS PATHOGENS IN THE CONTROL OF UDDER HEALTH IN DAIRY COWS

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Summary: The importance of systematic mastitis control in dairy herds is described through the presentation of data concerning mastitis occurrence and significance in modern milk production. Research was conducted during farm visits and by taking udder quarter samples from all lactating cows at the time of visit. Samples were taken before evening milking. Each sample was tested by the Zagreb mastitis test (ZMT) and examined bacteriologically by inoculation on aesculin blood agar. Identification of grown colonies was carried out using internationally accepted methodology. The obtained results were statistically analysed using the Stata 13.1 statistical package. Udder quarter samples from 385 cows were analysed. ZMT-positive reactions were found in 13.7% of all quarters. Mastitis pathogens were isolated from 175 (13%) of quarter samples. One hundred and forty five of 385 cows (37.3%) included in the study had at least one ZMT positive quarter or permanently lost (dried off) quarter. Mastitis pathogens were isolated in 106 of 363 cows (29.8%) with all four functional quarters. The most frequently isolated pathogens were *Staphylococcus aureus*, *Streptococcus* spp., *Trueperella pyogenes* and *Corynebacterium bovis*. There was no statistical difference in mastitis occurrence between the front and rear mammary quarters. The ZMT results and microbiological examination were moderately correlated (Kappa index = 0.4662).

Key words: cow; mastitis; mastitis pathogens; Croatia

Introduction

The occurrence of mastitis in a dairy herd above the tolerable limit causes multiple losses. One is lower milk prices or even confiscation of milk. Another, often overlooked, is lower milk production. Taking into account the costs of mastitis such as treatment, withdrawal of milk from the market and additional labour, it can be concluded that mastitis is a greater threat to the cow owner than to the animal's health.

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Among the economic losses due to mastitis, the dominant is reduced milk production, at 60% of total losses, followed by additional labour (16%), confiscated milk (9%), higher replacement rate (7%), lower market value of milk (4%), drugs (3%) and veterinary costs (1%) (1). Mastitis is an inflammation of the mammary gland of varying aetiology (2). However, mastitis is most commonly caused by numerous bacterial species. Hence, milk from mastitic glands is a possible threat to human health since it contains harmful bacteria and their toxins. Mastitis is the result of continuous competition between the pathogen and host defence mechanisms. Many

environmental factors favour bacterial success for the invasion of the mammary gland, such as: inadequate milking procedures, vacuum level of milking machine outside the recommended values, improper hygiene of milking machines, inadequate hygiene level of stalls and animals, poor microclimate conditions in stalls, insufficient feeding, etc. (1,3,4).

Milking technique is one of the key factors which are able to initiate infection and subsequent inflammation of the mammary gland. Either too short or too long milking time can be an initial point for mammary gland infections (5,6). Improper udder hygiene, milking of dirty or inadequately sanitized udders can also lead to mastitis. Furthermore, wounds on the udder and/or teat skin and anatomical failures of teat ends and sphincter are predisposing factors for mastitis. Supernumerary teats and fistulae serve as atria for the entry of bacteria into the mammary gland (7).

No other infectious disease can compare to mastitis in terms of the number of possible causative agents. The literature has recorded 150 or 200 different microbial species isolated from mastitis cases, predominantly bacteria, but also fungi or even monocellular achlorophylic algae. Despite the multitude of therapeutic agents and preventive measures, mastitis is still a predominant cause of losses in dairy production (8,9,10).

Mastitis appears in two forms: (i) subclinical with no clinical symptoms and (ii) clinical with one or more clinical signs such as: redness, oedema, pain, elevated local temperature and organoleptic changes in the milk (11). Regarding clinical appearance, mastitis occurs with a wide spectrum of symptoms, from no visible signs to extremely difficult symptoms which may lead to death of the infected animal. The clinical approach differentiates several types of mastitis regarding the intensity of infection, such as latent infection, catarrhal inflammation, acute phlegmonous or parenchymatous and oozing mastitis (12,13). Infective mastitis can be contagious, caused by a few of bacterial species such as *S. aureus, Streptococcus agalactiae* and *Corynebacterium bovis,* or environmental, caused by bacteria from the environment such as other streptococci, coliform bacteria, enterococci, etc. (14).

Despite many diagnostic methods available today, routine mastitis diagnosis is still mostly based on microbiological laboratory examination of properly taken udder quarter samples (15). Among different methods designed for screening in field conditions, mastitis testing using a reagent is inexpensive and accurate, and is easy to perform and interpret (16). Udder quarters giving a positive reaction should be submitted for microbiological examination (17).

This study emphasizes the importance of mammary gland health control in a systematic manner, showing the occurrence of mastitis in dairy herds, and the frequencies of isolated mastitis pathogens. Furthermore, it provides statistical evidence for the influence of quarter position (front/rear) on mastitis occurrence. Finally, it provides statistical evidence on the accordance between microbiological examination and field tests carried out using the Zagreb mastitis reagent.

Materials and methods

Animals

A total of 385 dairy cows from 15 farms were included in the study. Farms were located in Zagreb County, Sisak-Moslavina County and Karlovac County. Herd size ranged from 10 to 70 cows. Cows belonged to Simmental breed, Holstein-Friesian breed and their crosses.

Cows were kept in closed stalls, in smaller herds or free in larger farms. At all visited farms, cows were milked twice daily. Larger farms with a free stall system had stationary milking parlours, while at smaller farms, milking was performed at standing places either using a milking machine or by hand. The floor was either bedded with straw or sawdust in smaller herds or covered with rubber material in larger farms with a free stall system. Udder preparation for milking was performed by washing with warm water and drying with clean paper towels or individual cloths.

Sampling

Samples for examination were taken before the evening milking. After washing and drying, teat ends were disinfected with cotton swabs soaked in 70% ethanol. The first few streams were discarded. Approximately 10 mL of milk from each udder quarter was taken into sterile tubes. Samples were

transported to the laboratory on ice and stored at 4 °C until laboratory examination which was performed within 12 hours from sampling.

Each sample was examined using the Zagreb mastitis test (ZMT, Croatian Veterinary Institute, Zagreb, Croatia). ZMT is a field test intended for the identification of cows and quarters with abnormal udder secretions. It contains alkyl-aryl sulphonate which destroys cell membranes and induces DNA polymerisation. Hence, the intensity of the reaction in the mixture of equal aliquots of milk and reagent, i.e. consistency of the mixture, serves as an approximation for somatic cell number in the milk sample. Depending on the number of cells, the visible change in the mixture varies from no reaction (up to 3×10^5 cells per mL) to the formation of a gel with the consistency of egg white. Reactions in the mixture of ZMT and tested milk samples in this study were graded according to the manufacturer's recommendations as mild (visible threads in mixture), moderate (visible gel formation, but still liquid mixture consistency), and strong (formation of a gel consistency resembling egg white). Microbiological examination (MBE) was carried out according to the method recommended by the NMC (1999). Primary isolation was carried out by inoculation of samples onto nutrient agar (Merck, Germany) with 5% ovine blood and 0.1% aesculin and incubation at 37 °C. Inoculated plates were checked at 24-hour intervals. Grown colonies were stained according to Gramm (Merck, Germany), checked for catalase and oxidase production and further subcultured onto differential or selective media. Presumptive staphylococci colonies were subcultured onto Baird-Parker agar. Coagulase production in grown colonies was verified using 0.5 mL rabbit plasma (Merck, Germany). CAMP test was carried out to identify *Streptococcus agalactiae* among the presumptive streptococci. Gram-negative bacteria were subcultured onto MacConkey agar and Triple sugar iron agar (Merck, Germany). Pathogens were finally identified by biochemical profiling using Micronaut identification systems (Merlin Diagnostika, Germany) for gram-positive bacteria and gram-negative fermentative bacteria.

Statistical analyses were performed using the Stata 13.1 statistical package (Stata Corp, USA). Observed differences among udder quarters were examined using the chi-square test. Accordance between different diagnostic tests was tested by the Kappa statistics test.

Results

Results of ZMT for single quarters

Testing using ZMT encompassed 1540 udder quarters, of which 84.53% samples included somatic cells below the detectable level for mastitis reagents (< 3×10^5/mL). Among the samples with observed positive reaction, most were assigned to the mildest gradation (marked + or 1), which corresponds to a number of somatic cells from 3–5 × 10^5/mL. In the clinical examination performed during sampling, it was established that 29 udder quarters (1.88%) were permanently dysfunctional or lost for further milk production (Table 1).

Table 1: Data obtained by the ZMT in dairy cows

Reaction in ZMT	Dairy cows	
	n	%
Negative (-)	1300	84.53
Mild (+)	103	6.70
Moderate (++)	89	5.79
Strong (+++)	16	1.04
Pus	3	0.20
Nonfunctional quarter	29	1.88
Total	1540	100

Results of the MBE

In the MBE, 86.88% of all examined quarters were negative, *i.e.* none of the causative agents of mastitis were isolated. Among the isolated causative agents, the most frequent was *Staphylococcus aureus* in 69 (4.48%) of examined samples (Figure 1). The frequency of members of genus *Streptococcus* isolated from 37 samples (2.39%) was lower than that of *S. aureus* (Figure 2). Among other causative agents, a rather significant number of the isolated species was *Trueperella pyogenes* (previously: *Arcanobacterium*) from 26 (1.69%), *Corynebacterium* spp. from 25 (1.62%) and *Streptococcus uberis* from 18 (1.17%) of samples, whereas other causative agents were isolated only sporadically (Table 2; Figure 3).

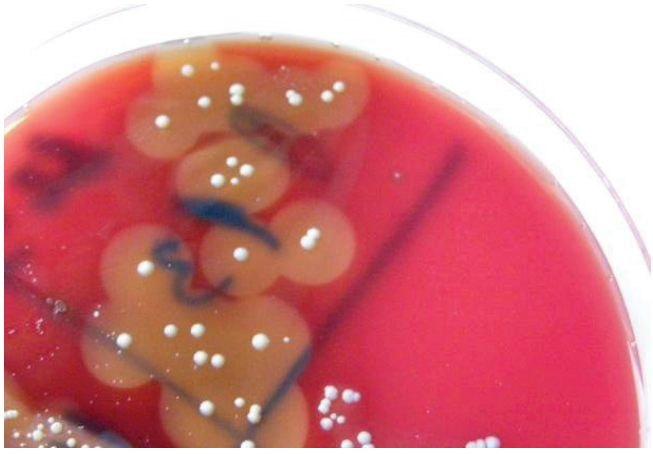

Figure 1: Colonies of *Staphylococcus aureus* on blood agar

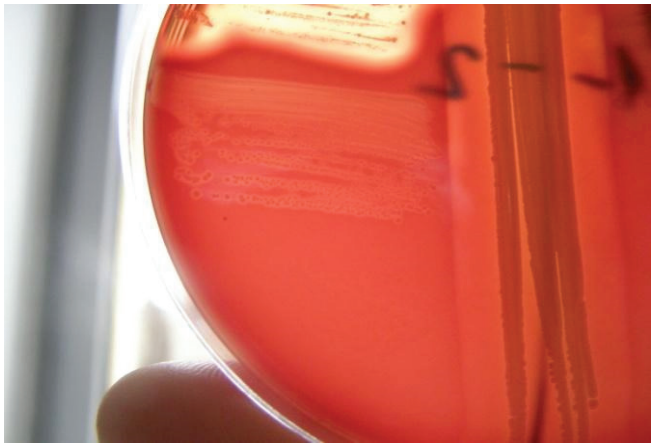

Figure 2: Colonies of *Streptococcus dysgalactiae* on blood agar

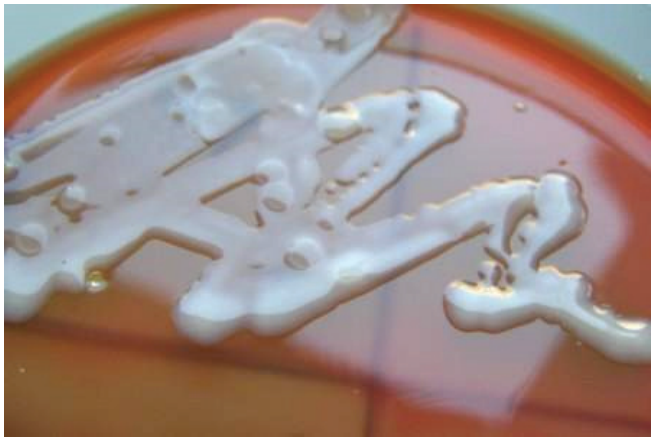

Figure 3: Colonies of *E. coli* on blood agar

Table 2: Microbes isolated by the MBE from dairy cows with mastitis

Isolated organisms by MBE	Dairy cows	
	n	%
Staphylococcus aureus	69	4.48
Trueperella pyogenes	26	1.69
Corynebacterium spp.	25	1.62
Streptococcus uberis	18	1.17
Streptococcus spp.	11	0.71
Enterococcus spp.	5	0.32
Streptococcus dysgalactiae	5	0.32
Staphylococcus spp.(CNS)	4	0.26
E. coli	3	0.19
Streptococcus agalactiae	3	0.19
Klebsiella spp.	1	0.06
Pasteurella spp.	1	0.06
Proteus sp.	1	0.06
Enterobacter sp.	1	0.06
Pseudomonas sp.	1	0.06
Yeasts	1	0.06
Non-functional quarter	27	1.75
No growth	1338	86.88

Results of examinations according to number of dairy cows

Of total 385 cows examined, no clinical changes were observed in 240 (62.34%) cows. Clinical changes in one quarter were recorded in 17.66% of examined cows, and in two quarters in about 8% of cows. In 26 cows (6.75%), quarters were found to have chronic damage, resulting in a total loss of function. Of the 363 cows with functional all udder quarters, 257 (70.8%) had no quarters infected. Among the microbiologically positive cows, the highest proportion had one infected quarter (61 cows or 16.8%) (Figure 4).

The proportion of quarters that showed no reaction to the ZMT, *i.e.* in which the number of cellular elements was below the level of detection by ZMT reagent, was between 82.6% and 86.16%, depending on the anatomical position of the quarter. The observed difference in frequency of a positive reaction between front and rear quarters was not statistically significant ($P>0.05$) (Table 3).

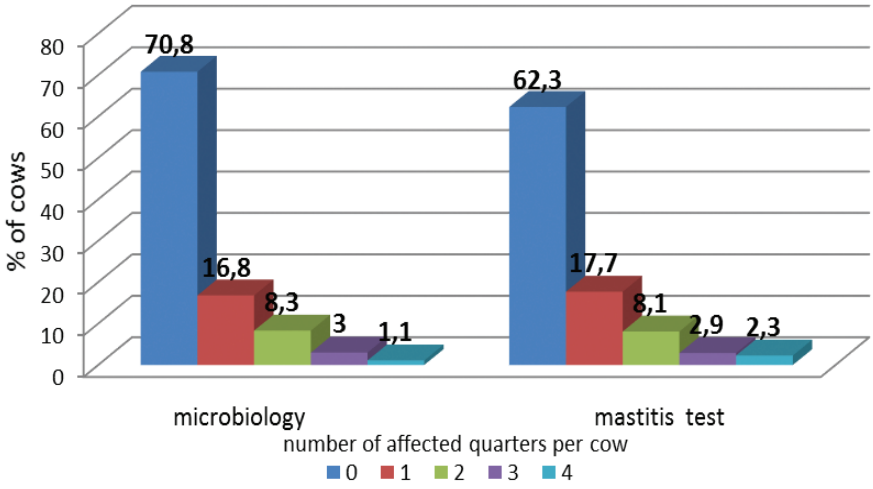

Figure 4: Comparison of the results obtained by MBE and ZMT per cow

Table 3: Intensity of reaction in ZMT per single quarters of dairy cows

Reaction in ZMT	I quarter		II quarter		III quarter		IV quarter		Total		*P*-value
	n	%	n	%	n	%	n	%	n	%	
Negative (-)	330	86.2	328	85.2	318	82.7	324	84.2	1300	84.4	0.32
Mild (+)	26	6.8	21	5.4	28	7.3	28	7.3	104	6.7	
Moderate (++)	16	4.2	20	5.2	32	8.3	21	5.4	89	5.7	
Strong (+++)	4	1.0	4	1.0	2	0.5	6	1.6	16	1.0	
Pus	1	0.3	1	0.3	-		1	0.3	3	0.2	
Nonfunctional quarter	8	1.6	11	2.9	5	1.3	5	1.3	27	1.8	

Table 4: Data obtained by MBE per quarter of dairy cows

Udder quarter	None of agents isolated		Isolated causative agent		*P*-value
	n	%	n	%	
I	337	88.9	42	11.1	0.58
II	337	90.1	37	9.9	
III	332	87.4	48	12.6	
IV	332	87.3	48	12.6	
Total	1338	88.4	175	11.5	

Table 5: Congruence between ZMT and MBE results

ZMT result	MBE result		Total
	Negative	Positive	
Negative	1228	72	1300
Positive	108	103	211
Kappa index	0.4661		

The proportion of udder quarters from which a causative agent of mastitis was isolated ranged from 9.9% (in the II quarter) to 12.6% (in III and IV quarters). The observed differences in the frequency of infections between different quarters were not statistically significant (Table 4). Accordance between the data obtained by ZMT and MBE was found to be moderate, with Kappa index of 0.466 (Table 5).

Discussion

Though modern milk production includes a spectre of preventive measures for the prophylaxis of mastitis, this disease is still the cause of the highest economic losses in dairy cattle breeding. There are many reasons why the causative agents of mastitis are successful in infecting the udder. Very few of infectious diseases are comparable to mastitis in terms of potential numbers of causative agents. According to the literature, more than 150 different microbial species have been indicated as causative agents of mastitis. These belong to a wide range of taxonomic categories. The most frequent are: bacteria, fungi and even monocellular aclorophylic algae [18,19].

Most other infections occur by the galactogenic route, *i.e.* by the entry of the causative agents into the udder through the teat canal. The teat canal is open during milking and stays partially open for a short time after milking. This period could be prolonged if the milking machine is not technically functional. The mucosae of the teat canal may even prolapse in cases when the vacuum pressure for milking is higher than recommended. Hence, mastitis is very frequently a consequence of the technical malfunction of milking machines. Furthermore, many cows share same the milking unit. When the disinfection procedure is not performed after the milking of each cow, the possibility of infection transmission increases even if a single cow in the milking order is infected. In other words, it is known that many causative agents are present in the milking environment and their transmission from infected to non-infected animal during milking is possible if farmers do not follow the standard hygienic procedures. Furthermore, cows in one phase of lactation produce a high milk yield, which demands increased feed intake and thus increased energy and entering into a negative energy balance (NEB). In such cases, cows use their own body reserves in order to produce milk yield in accordance to their genetic potential. During the period of NEB followed by increased metabolic demand, cows are more susceptible not only to mastitis, but also to other, particularly metabolic disorders such as ketosis (20,21,22).

In the present study, more than 15% of the examined udder quarters showed signs of disturbance in secretion in testing with ZMT. In comparison with a similar studies, the proportion of quarters with a positive reaction was extremely decreased. Namely, in the studies conducted in 1997 and 2004, the proportion of positive reactions was 34% and 28%, respectively (23). These studies were performed using similar methodology but in other parts of the country and at the time of enforcement of new legislation regarding the calculation of raw milk prices. Accordingly, it can be assumed that the decreased number of quarters with positive reaction to ZMT are the result of the implementation of preventive measures directed towards udder health and thus to the hygienic quality of milk.

By MBE, the causative agents of mastitis were isolated in 175 (13%) of tested samples. The most common was *S. aureus* (4.5%), as previously reported in Croatia and worldwide (24,25). However, the total number of infected quarters by this agent was less than half in comparison to the previous studies (11%). This finding supports the assumption of improved overall hygienic standards in primary milk production. Namely, at the farms visited, it was observed that the teat was routinely dipped into disinfectant following milking. This procedure was applied without exception at all farms with a milking parlour. It should be emphasized that teat dipping following milking is considered to be an effective measure to control and reduce contagious mastitis (26).

The second typical contagious agent, *Streptococcus agalactiae*, was isolated from less than 1% of tested samples. In all performed tests, the frequency of this agent was less than 3%. However, isolation of this agent, even in low numbers, indicates possible errors in mastitis prevention at dry-off. Namely, this agent survives almost exclusively in the udder, while it is very rapidly destroyed in the environment; therefore, this is one of the rare causative agents which could be totally eradicated in the herd. In thise regard, the use of antibiotics in dry-off with other preventive

measures is effective in dairy cattle herds (27).

More often than in previous studies, bacteria from the genus *Corynebacterium* and *Trueperella pyogenes* were isolated. *Corynebacterium bovis* is a mild pathogen, but it colonizes the teat canal and occasionally induces a small increase of somatic cell number and lowers milk yield. In this regard, infections caused by these bacteria are mild and more difficult to detect (15).

With respect to previous studies, more cases of *T. pyogenes* were recorded in the present study. This causative agent very often causes purulent inflammations, and in addition to mastitis, it also induces changes in other organs and tissues. It is related to summer mastitis, which is a more significant health problem in the northern Europe. It is assumed that flies play an important role in the spread of this pathogen from infected to non-infected animals. Since the present study was performed in late winter and early spring when flies are not active, and we have observed an increased frequency of this agent in comparison to previous studies, it would be advisable to enhance monitoring in dairy cow herds during spring and summer, in order to avoid the possible spread of this agent by the increased activities of flies (28).

Significant numbers of *Streptococcus uberis* were isolated. This pathogen most frequently infects cows from the environment, but with a higher frequency in the early dry-off period than during other phases of lactation. In this regard, in order to prevent mastitis caused by this agent, it is crucial to ensure a clean and dry environment, particularly in the dry-off period. The frequency of other causative agents was relatively low and sporadic and the obtained results were similar to data reported previously. Some of these agents may infect the udder relatively frequently with a lethal outcome, such as in the case of *E. coli* infection. The reason for low prevalence in such systematic studies is that infections are of a short duration. The chance for isolation of *E. coli* from an affected udder is greater when the sample for examination is taken immediately after the appearance of the first mastitis symptoms. It is assumed that live bacteria are no longer present in udder secretions after 5 to 6 hours from the first appearance of symptoms. Clinical disorders appear due to endotoxin, which is released from dead bacterial cells (29).

When considering the results of ZMT and MBE per animal, it is evident that a relatively high proportion of the dairy cows were affected by the causative agents. More than one-third of animals showed a positive reaction to ZMT in at least one udder quarter. According to some authors, up to 50% of cows are affected by mastitis. The proportion of cows with the presence of pathogens within at least one udder quarter was almost 30% in this study. Almost half of the cows, from which the pathogens were isolated, had more than one udder quarters infected. When mastitis is considered from this aspect, the status of its spread, significance for the modern milk industry and importance of systematic control of mastitis in dairy cow herds (2) becomes clearer.

Regarding the intensity of reaction observed in ZMT testing, the highest proportion of reactions were of the lowest degree that could be detected by this method. This fact supports the statement that the mastitis prevention is at a relatively high level in the examined herds. Farmers promptly react to changes in secretion and thus very few cases end with a chronic form of mastitis with extreme reactions (30). The detection of mastitis is performed by the mastitis test, and in some farms with milking parlours, the milking machine measures the electric conductivity of the milk. Namely, in inflamed quarters, at the beginning of the milking process, the milk's electric conductivity is altered due to an increased concentration of chlorides, which are characteristic of the early phases of the inflammatory process (31).

Some studies have reported differences in mastitis frequencies between the front and rear udder quarters. It is assumed that the reason for more frequent infection of rear quarters than front quarters is in their anatomical position and higher exposure to trauma. In the present study, differences were found among quarters and also in the intensity of reaction between quarters, although they were not statistically significant ($P>0.05$). Similarly, the differences in the frequency of isolation of the causative agent between the quarters were also not statistically significant ($P>0.05$) (25).

MBE is often used as the gold standard for validation of reliability of other diagnostic tests. In this study, we compared the results of the ZMT with those of the MBE by means of the Kappa test. Accordance between these two methods was found to be moderate (0.4661). Although the result of the ZMT is a moderate indicator of inflammation, it should be pointed out that an increased number of cellular elements in milk

might be a consequence of trauma or other causes. A reliable scientific approach for more accurate determination of inflammation status of the udder quarters requires more frequent milk sampling during the same milking or more sampling at regular time intervals.

When considering the importance of the mastitis diagnosis, its public health and economic significance should be taken into account. The number and variety of possible causative agents, differences in their spread and survival in the environment are reasons for the use of the MBE as an essential method. Some of the causative agents may pose risk for human health. Furthermore, bacterial resistance to antibiotics should not be overlooked. In other words, bacteria are capable of acquiring and transferring antibiotic resistance to other, taxonomically related and unrelated bacteria. Since the susceptibility test is an essential part of the MBE, the importance of this method in the diagnostics of mastitis and in monitoring trends in the appearance and spreading of resistance to antibiotics is evident (32).

Among mastitis cases, subclinical infections are dominant and the only sign of infection is an elevated somatic cell count. Hence, problematic herds are often revealed during routine controls of somatic cell counts at the purchase of raw milk. Milk with a high somatic cell count is unacceptable for further processing and sale, which increases the total costs of mastitis. However, lower milk yield in cows with infected udders is the predominant cause of economic loss due to mastitis. Picinni et al. (33) calculated that the individual loss per cow in the herd due to mastitis is between 55 and 113 euros.

Conclusions

More than one-third of cows in the present study had changes in at least one udder quarter. The degree of change ranged from the mildest degree that could be detected by the ZMT, to permanently dry-off and loss of quarters for further milk production. About 30% of examined cows in this study had at least one udder quarter infected. The most frequently isolated causative agents of mastitis were *S. aureus* and streptococci, although in a lower degree than in similar previous studies. Concordance of the data obtained by the methods applied in this study was moderate. The ZMT proved to be a rapid, simple and low cost means of mastitis detection in dairy cows. However, it should be supported with data obtained from the MBE in order to select the most effective therapy and to prevent the occurrence of antibiotic resistance.

Conflict of interest

Authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

1. Rupić V. Zaštita zdravlja domaćih životinja. 3. Fiziologija i patologija reprodukcije. Zagreb : V. Rupić, 2010: 273–99.

2. Radostits OM, Gay CC, Blood DC, et al. Veterinary medicine: textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. London : Saunders, 2000: 603–700.

3. Bačić G. Dijagnostika i liječenje mastitisa u goveda. Zagreb : Veterinarski fakultet, Sveučilište, 2009: 55–70.

4. Turk R, Piras C, Kovačić M, et al. Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. J Proteomics 2012; 75: 4412–28.

5. Mein GA, Reinemann DJ, Schuring N, et al. Milking machines and mastitis risk: a storm in a teatcup. In: Proceedings of the 43rd Annual Meeting of the National Mastitis Council. Ontario, 2004: 176–88.

6. Mein GA. The role of the milking machine in mastitis control. Vet Clin North Am Food Anim Pract 2012; 28: 149–390.

7. Neijenhuis F. Teat condition in dairy cows. Dissertation. Utrecht : Faculty of Veterinary Medicine, 2004.

8. Steeneveld W, Van Werven T, Barkema HW, et al. Cow-specific treatment of clinical mastitis: an economical approach. J Dairy Sci 2011; 94: 174–88.

9. Stepanić M, Benić M, Habrun B, et al. Uzročnici mastitisa niske pojavnosti. Vet Stn 2014; 44: 41–7.

10. Aebi M, Van der Borne BH, Raemy A, et al. *Mycoplasma bovis* infection in Swiss dairy cattle: a clinical investigation. Acta Vet Scand 2015; 57: e10 (11 pp.)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347908

11. Heinze J, Donat K, Brandt HR, et al. Subclinical staphylococcal intramammary infections: within-herd prevalence and effects on milk yield and somatic cell counts in Thuringian dairy herds. Berl Munch Tierarztl Wochenschr 2015; 128: 61–9.

12. Makek Z. Osvrt na dijagnostiku, terapiju i preventivu upala mliječne žlijezde u krava. Mljekarstvo 1995; 45: 275–82.

13. Stoliuk V, Valchuk O. Mastitis in Ukrainian cows: effective ways to solve the problem. Int Dairy Top 2011; 10: 13–7.

14. Gračner D, Bedrica Lj, Cergolj M, et al. Haptoglobinspielel in Blut und Milch von Kuhen mit einer Staphylokokkenmastitis. Tierarztl Umsch 2006; 61: 636–41.

15. National Mastitis Council. Laboratory handbook on bovine mastitis. Rev. ed. Madison, WI : National Mastitis Council, 1999.

16. Sargeant JM, Leslie KE, Shirley JE, et al. Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation. J Dairy Sci 2001; 89: 2018–24.

17. Pravilnik o pregledu sirovog mlijeka namijenjenog javnoj potrošnji. NN 2010; 110/10: 2906 (22. 9. 2010)

18. Möller A, Truyen U, Roesler U. *Prototheca zopfii* genotype 2: the causative agent of bovine protothecal mastitis? Vet Microbiol 2007; 10: 370–4.

19. Piepers S, De Meulemeestera L, De Kruif A, et al. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J Dairy Res 2007; 74: 478–83.

20. Wathes DC, Fenwick M, Cheng Z, et. al. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 2007; 68: S232–41.

21. Kočila P, Janžek A, Gračner D, et al. Vergleich von Progesteronkonzentrationen, Energiebilanzkennwerten und körperlicher Verfassung bei Milchkühen mit verschiedener Milchleistung im Puerperium. Tierärztl Umsch 2013; 68: 266–74.

22. Đuričić D, Vince S, Gračner D, et al. Vergleich von zwei Methoden zu Bestimmung der Prävalenz subklinischer Ketose bei Milchkühen in Nordwestkroatien. Tierärztl Umsch 2015; 70: 55–9.

23. Benić M. Učestalost mastitisa prije i poslije donošenja pravilnika o kakvoći svježeg sirovog mlijeka. Vet Stn 2005; 36: 233–8.

24. Pavlak M, Benić M, Cvitković D, et al. Epidemiological data of intramammmary infection in cattle: a quantitative analysis of published data. In: Proceedings of 16th Congress of the Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum). Zadar, Croatia. Zagreb : Veterinarski fakultet Sveučilišta, 2008: 97–112.

25. Maćešić N. Učinkovitost pojedinih metoda zasušivanja krava. Disertacija. Zagreb : Veterinarski fakultet Sveučilišta, 2010.

26. Hogan JS, White DG, Pankey JW. Effects of teat dipping on intramammary infections by Staphylococci other than *Staphylococcus aureus*. J Dairy Sci 1987; 70: 873–9.

27. Keefe GP. *Streptococcus agalactiae* mastitis: a review. Can Vet J 1997; 38: 429–37.

28. Andrews AH, Blowey RW, Boyd H, et al. Bovine medicine: diseases and husbandry of cattle. 2nd ed. Oxford : Blackwell Science, 2008: 334.

29. Wenz JR, Barrington GM, Garry FB, et al. Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. J Am Vet Med Assoc 2004; 219: 976–81.

30. Đuričić D, Samardžija M, Grizelj J, et al. Effet du traitement intramammaire des mammites subcliniques pendant la lactation en élevages bovins laitiers au nord-ouest de la Croatie. Annal Méd Vét 2014; 159: 121–5.

31. Norberg E, Hogeveen H, Korsgaard IR, et al. Electrical conductivity of milk: ability to predict mastitis status. J Dairy Sci 2004; 87: 1099–107.

32. Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 2011; 4: 236–43.

33. Piccinini RE, Binda L, Zecconi A. Prevalence study on bulk milk tank cultures in 1000 dairy herds in Lombardia (Italy). In: 42nd National Mastitis Council Annual Meeting. Forth Worth, Texas, 2003: 396–7.

MIKROBIOLOŠKO SPREMLJANJE POVZROČITELJEV MASTITISA OB NADZORU ZDRAVJA VIMENA PRI KRAVAH MOLZNICAH

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Povzetek: Pomen sistematičnega nadzora mastitisa pri čredah krav je opisan s predstavitvijo podatkov o pojavnosti mastitisa in njegovemu pomenu za sodobno proizvodnjo mleka. Raziskava je bila opravljena v obdobju obiskov kmetij ob odvzemu vzorcev iz vsake vimenske četrti vsem kravam v laktaciji med obiski. Vzorci so bili odvzeti pred večerno molžo. Vsak je bil testiran z zagrebškim »mastitis testom« (ZMT) in za bakteriološki pregled nasajen na krvni agar z eskulinom. Identifikacija zraslih kolonij je bila izvedena s pomočjo mednarodno sprejete metodologije. Dobljeni rezultati so bili statistično obdelani s pomočjo statističnega paketa Stat 13.1. Analizirani so bili vzorci vimenskih četrti 385 krav. ZMT-pozitivne reakcije so bile ugotovljene v 13,7 % vimenskih četrtih. Povzročitelji mastitisa so bili ugotovljeni v 175 vzorcih (13 %) četrti. Od 385 krav, vključenih v raziskavo, jih je 145 (37,3 %) imelo vsaj eno ZMT pozitivno vimensko četrt ali trajno osušeno vimensko četrt. Povzročitelji mastitisa so bili izolirani pri 106 od 363 krav (29,8 %), iz vseh štirih funkcionalnih četrti. Najpogosteje izolirani povzročitelji so bili *Staphylococcus aureus*, *Streptococcus spp.*, *Trueperella pyogenes* in *Corynebacterium bovis*. Statistično značilnih razlik v pojavljanju mastitisa med sprednjo in zadnjo vimensko četrtjo ni bilo. Rezultati ZMT in mikrobiološki pregled sta v zmerni korelaciji (indeks Kappa = 0,4662).

Ključne besede: krava; mastitis; povzročitelji mastitisa; Hrvaška