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Original article

The prevalence of some microorganisms in cows' milk with regard to lactation number, lactation period and somatic cell count

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Abstract

The aim of this study was to assess the effect of lactation number, lactation stage and somatic cell count (SCC) on the presence of pathogenic or opportunistic pathogens in cow milk. A total of 1712 milk samples were collected from the udder quarters of 428 lactating Holstein breed cows for bacteriological examination. Somatic cell count was taken from the controlled bovine records. The cows were divided into four groups according to the lactation number (*viz.* lactation numbers 1, 2, 3, 4 and above) and into three groups according to the lactation month (*viz.* 1-4, 5-8, 9 months and above). The statistical analysis was performed by SPSS 27.0 software (SPSS Inc., Chicago, Illinois, USA). Frequencies of microorganisms were calculated by determining their confidence intervals (Wilson Confidence Interval 95%, CI). Various farm pathogens were identified: CNS (Coagulase negative staphylococci*), S. aureus, Enterococcus* spp., *Str. agalactiae, E. coli*. It was found that CNS and *S. agalactiae* increased with somatic cell count, lactation number and lactation stage. *E. coli* increased at the end of the lactation stage ($p \leq 0.05$). *Enterococcus* spp., count in milk differed significantly between cows in lactations 1 and 4 and older ($p \leq 0.05$). Pathogen number also increased with milk fat, but decreased with increased protein content ($p \leq 0.01$).

Keywords: cattle, lactation, mastitis, milk quality, pathogens, production

Introduction

Mastitis is a common endemic disease in dairy herds in many different countries and hence it is an important cause of economic loss and less efficient milk production (Morales-Ubaldo et al. 2023). It poses a challenge to the entire dairy industry (Zhang et al. 2016, Cheng et al. 2019, Cheng and Han 2020). When monitoring cows for subclinical mastitis, it is necessary to identify the potential pathogens and determine the somatic cell count in milk (Petzer et al. 2017). Watts (1988) estimated that about 150 species of microorganisms are responsible for mastitis, including many human pathogens. Bacterial mastitis has been associated with a number of pathological agents, including *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae,* coagulase negative staphylococci (CNS), *Streptococcus uberis*, the coliforms: *Escherichia coli, Enterobacter, Klebsiella* species*, Corynebacterium bovis*, *Mycoplasma* spp.*, Pseudomonas* spp. and *Enterococcus faecalis*, as well as yeasts and other fungi (Cobirka, et al. 2020). Among these, the most frequently mentioned pathogens, apart from *S. agalactiae* and *S. aureus,* are also *Escherichia coli, Streptococcus uberis, Klebsiella* spp.*,* coagulase-negative *Staphylococcus* species *(CNS)*, and *Prototheca* spp. (Jagielski, et al. 2019, Rifatbegović et al. 2024). In a Lithuanian cow herd, the most prevalent mastitis pathogens were found to be *Staphylococcus aureus* (15.25%) and *Streptococcus agalactiae* (4.80%) (Antanaitis et al. 2021). Mastitis is a multifactorial disease, with breed, age, parity, herd size, period of lactation, hygiene and weather known to influence the subclinical form (Cobirka et al. 2020). Indeed, studies have examined the influence of intramammary infection and microorganism type on somatic cell count (SCC) in dairy cow milk, as well as age, parity, stage of lactation, and milk production level (Sumon et al. 2020).

Environmental mastitis is usually caused by *E. coli.* This pathogen enters the mammary gland during early lactation and it can cause lethal infection if it is left untreated *(*Cobirka, et al. 2020). The course of infection usually starts as the subclinical form and then develops into clinical mastitis during early lactation, continuing for more than 100 days into the milking period (Cheng et al. 2020). Indirect evidence shows that younger cows in their first lactation period are more resistant to contagious causative agents, with 12–15% of first-lactation cows being found to be already infected with *S. aureus* (Goli et al. 2012). Research indicates that cows older than seven years are at 13-times greater risk of udder infection than cows in their first to third lactation, and primary cows are two- to three-times more susceptible to CNS than older cows (Souza et al. 2016). Subclinical mastitis was found to be shorter in early lactation than late lactation in primiparous cows; however, no such difference in duration was noted for the clinical form in multiparous cows (Breen et al. 2009). In addition, during early lactation, primiparous cows tended to suffer subclinical mastitis earlier than the multiparous cows; however, in late lactation, subclinical mastitis occurred later in primiparous than multiparous cows (Breen et al. 2009). The age of cows also influences the occurrence of mastitis (Kayano et al. 2018), with older animals being more susceptible to mastitis than younger ones. With advancing age, the udder becomes more pendulous, thus increasing the risk of injury, its glandular tissue becomes sensitive to inflammation, and older cows have poorer defence mechanisms than younger ones (Elmaghraby et al. 2017). The mammary epithelium can also demonstrate greater permeability in older cows caused by previous inflammatory states, even after successful treatment (Kibebew 2017). The aim of the present study was to determine the possible relation between the prevalence of *S. aureus*, *Str. agalactiae* and *E.coli* amongst other opportunistic pathogens and lactation number, lactation period, somatic cell count and milk composition parameters in cows.

Materials and Methods

Research material

The study involving 428 lactating Holstein cows was conducted from November 2022 until February 2023 on the farm. The animals were kept loose in cold-type barns on rubber mats all the year round. The cows were fed a complete diet that met their physiological needs: they received a total mixed diet based on grass silage, maize silage, hay and concentrate to meet the nutritional requirements of a 600 kg cow yielding about 20 kg of milk per day. All animals were kept in accordance with established hygiene requirements.

The cows were milked twice a day in a side-by-side milking parlour.

Sampling

A total of 1712 milk samples were taken for bacteriological examination from the udder quarters of 428 cows over four months, with one sample taken from each cow once a month. There were no further specifications regarding udder health parameters such as the incidence of clinical mastitis or detected pathogens.

Composite milk sample from all quarters of each analysed cow was collected into sterile tubes at the end of the milking process (during test milkings) and deli-

vered to the laboratory for immediate bacteriological evaluation. The research was performed in accordance with the provisions of the Law on Animal Welfare and Protection of the Republic of Lithuania, No XI-2271 (2012).

Identification

Bacteriological pathogens were identified according to standard operating procedure SDP 5.4.4.B.6 "Fundamental mastitis-causing bacteria evaluation in milk" developed by "Laboratory and field handbook on bovine mastitis" (Adkins et al. 2017).

Milk samples were examined using common bacteriological methods. Each of 10 µL milk samples was streaked on Blood agar (Colombia agar with 5% sheep blood, E&O Laboratories Limited, Scotland), Modified Edwards Medium (E&O Laboratories Limited, Scotland) and MacConkey agar (Liofilchem, Italy). Inoculated media were incubated at 37°C under aerobic conditions. Possible growth on plates were observed at 18 and 24 hours (48 hours for streptococcus and staphylococcus). On primary culture media bacteria identified tentatively. It was based on colony morphology (size, pigment production, texture), haemolysis on blood agar, bacteria morphology (Gram staining) and catalase test. Typical colonies were selected for further identification.

Staphylococcus aureus confirmed by mannitol fermentation and coagulase production (Staphytect Plus™ Latex Agglutination Test, Thermofisher, USA). *Streptococcus agalactiae* was confirmed by Gram staining, catalase test, presence of haemolysis on blood agar, growing on Edwards agar (E&O Laboratories Limited, Scotland). The CAMP, bacitracine, optochine and additional biochemical tests such acid formation from inulin, lactose, mannitol, salicin, trehalose, sorbitol and serotyping were provided also. Enterococcus spp. identified by growing on MacConkey, Slanetz Bartley (Liofilchem, Italy) agar, Gram staining, oxidase (Becton Dickinson, USA), catalase, CAMP tests and esculin hydrolysis.

E.coli isolated on MacConkey (Liofilchem, Italy) and Blood agar (E&O Laboratories, Scotland, UK). Plates incubated for 18-24h, at 35°C. *E.coli* confirmed by biochemical tests (Microbact GNB Kit, Thermo Scientific, USA). Other bacteria considered as Enterobacteria (associative to coliform bacteria). They confirmed by growing with typical colonies on MacConkey, Endo, Billiant green agar, Gram staining, oxidase, catalase, indole and H2 and biochemical tests.

Determination of somatic cells and milk composition parameters

Data concerning somatic cell count and milk composition indicators were obtained from monthly test milkings from November 2022 until February 2023. Fat, protein and lactose content was determined by LactoScope FTIR infrared instrument (Perten Instruments, Stockholm, Sweden). Somatic cell number was counted by a Somascope heavy-duty counter-measurer (Perten Instruments, Stockholm, Sweden) which operates by the fluoro-opto electronic method. Milk analysis was performed in the *Joint Stock Company* Pieno Tyrimai – accredited central milk testing laboratory (Kaunas, Lithuania).

The cows were divided into three groups based on SCC count: Group I with SCC up to 200 000 cells/mL, Group II with 200 000 to 400 000 cells/mL and Group III with more than 400 000 cells/mL. In addition, they were divided into four groups according to lactation number: first lactation (n=184), second lactation (n=96), third lactation (n=68), and fourth lactation and above (n=80). The lactation of cows lasted on average 331 days. They were also divided into three groups based on lactation month: months 1-4 (n=136), 5-8 (n=144), 9 and over (n=148).

Statistical analysis

The statistical analysis was performed using SPSS 27.0 (SPSS Inc., Chicago, IL). The frequencies of the pathogens were calculated based on their confidence intervals (Wilson Confidence Interval 95%, CI). The influence of lactation, lactation period, somatic cell count, milk fat content, milk protein content and milk lactose content on pathogen frequency was determined using the Chi-squared test, or the Fisher's Exact Test for Count Data for small counts after constructing a table. The data acquired from the two previous stages were then compiled and further investigated using a logistic regression model. Differences were considered statistically significant at $p \leq 0.05$.

Results

Our results indicated that from 1712 milk samples collected from lactating cows, about 82% were infected with at least one pathogenic species. Meanwhile in our present study, the most common pathogens detected in the milk were CNS (46.7% of milk samples, n=800); $p \leq 0.001$), while the lowest was *S. aureus*, detected in only 0.23% of all milk samples ($p \leq 0.001$).

Figure 1 describes the relationships between the various indicators known to influence pathogen num-

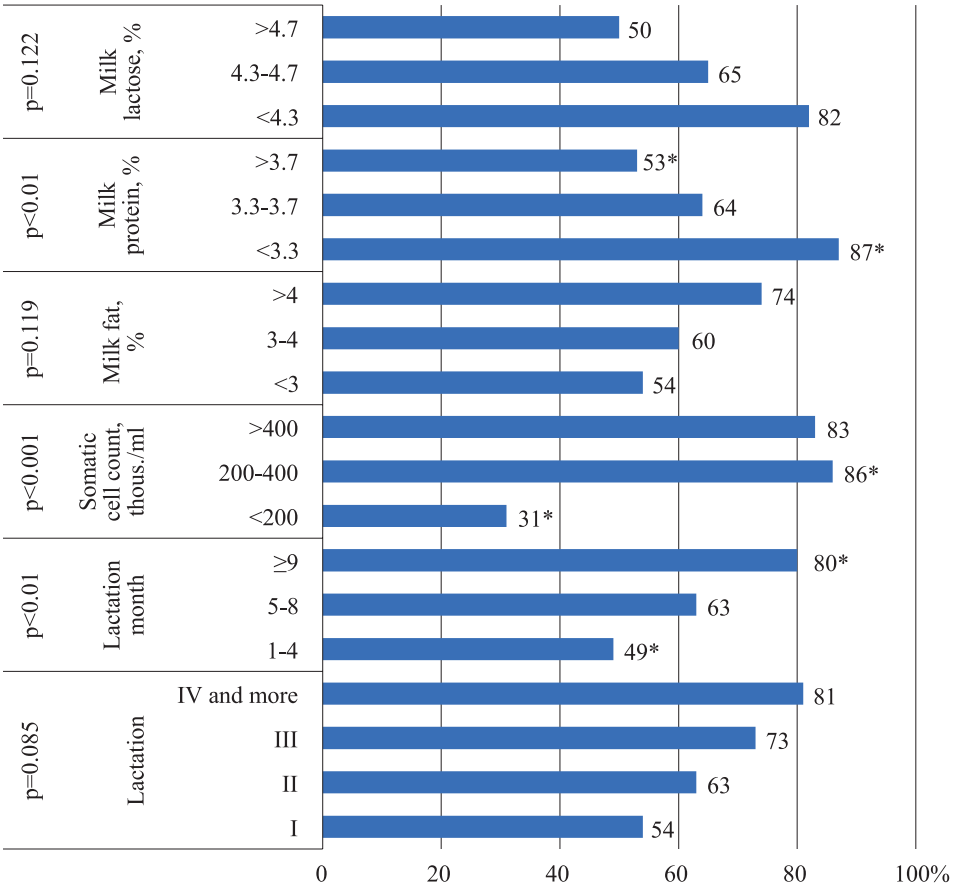

Fig. 1. Total prevalence of pathogens (%) isolated from cows' milk depending on studied indicators.
* Statistical significance between values within a group.

Table 1. Prevalence of pathogens isolated from milk depending on lactation number, given as percentages (%).

Lactation number	n	CNS	*Enterococcus* spp.	*S. agalactiae*	*E. coli*	*S. aureus*
I	184	39.1	4.3	8.7	10.9	
II	96	41.7	12.5	12.5	8.3	1.0
III	68	52.9	29.4	11.8	17.6	
IV and over	80	65.0	20.0	15.0	5.0	
		p=0.242	p≤0.05	p=0.853	p=0.695	p=0.570

ber. The total prevalence of pathogens (%) detected in milk was found to increase with cow lactation number, although not significantly. In addition, pathogen count was found to increase with the lactation period (p≤0.01), and varied according to somatic cell count (p≤0.001).

Prevalence of CNS, *Enterobacteria* spp., *S. agalactiae, E. coli, S. aureus* isolated from milk depending on lactation number was presented in Table 1. Lower pathogen counts, except for *E. coli*, were recorded in the first lactation cows compared with older lactation cows. *Enterobacteria* spp count was also found to increase with lactation number (p≤0.05).

The results relating to the number of separate bacteria species isolated from milk from the analyzed lactation periods are presented in Table 2. It was found that the counts of CNS, *S. agalactiae* and *E. coli* increased with lactation period, but the highest *Enterococcus* spp. count was detected during the first months of lactation.

Generally, no trends were found regarding bacteria which can lead to mastitis in different lactations during different lactation periods (Table 3). However, for all lactation periods, *E. coli* was not detected at the beginning of lactation, and was more frequently observed at the end. Also, a significant increase in CNS was observed at the end of the first lactation compared to the middle (p=0.008).

Table 4 present results of pathogens prevalence depending on somatic cell count. Results show that *Enterococcus spp.* and *Strep. agalactiae* were mostly

Table 2. Prevalence of pathogens isolated from milk depending on lactation period, given as percentages (%).

Lactation period	N	CNS	*Enterococcus* spp.	*Strep. agalactiae*	*E. coli*	*Staph. aureus*
1-4 month	136	35.3	17.6	5.9		
5-8 month	144	41.7	13.9	11.1	11.1	0.7
9 and over	148	62.2	8.1	16.2	18.9	
		p=0.063	p=0.485	p=0.474	p≤0.05	p=0.654

Table 3. Prevalence of pathogens isolated from milk depending on lactation number and lactation period, given as percentages (%).

Lactation	Lactation period	CNS	*Enterococcus* spp.	*Strep. agalactiae*	*E. coli*	*Staph. aureus*	
I	1-4 month	23.1	15.4	0.0	0.0	0.0	p=0.077
	5-8 month	15.4	0.0	15.4	7.7	0.0	p=0.540
	9 month and over	65.0	0.0	10.0	20.0	0.0	p≤0.001
		p=0.008	p=0.151	p=0.555	p=0.251	p=1	
II	1-4 month	50.0	12.5	12.5	0.0	0.0	p=0.056
	5-8 month	45.5	9.1	9.1	18.2	2.3	p=0.261
	9 month and over	20.0	20.0	20.0	0.0	0.0	p=1
		p=0.656	p=0.999	p=0.999	p=0.681	p=0.999	
III	1-4 month	20.0	20.0	0.0	0.0	0.0	p=0.999
	5-8 month	71.4	28.6	14.3	14.3	0.0	p≤0.05
	9 month and over	60.0	40.0	20.0	40.0	0.0	p=0.491
		p=0.338	p=0.999	p=0.999	p=0.434	p=1	
IV and older	1-4 month	50.0	25.0	12.5	0.0	0.0	p=0.062
	5-8 month	60.0	40.0	0.0	0.0	0.0	p≤0.05
	9 month and more	85.7	0.0	28.6	14.3	0.0	p≤0.01
		p=0.425	p=0.307	p=0.582	p=0.600	p=1	

Table 4. Prevalence of pathogens isolated from milk depending on somatic cell count, values given as percentages (%).

SCC, thousand/ml	CNS	*Enterococcus* spp.	*Strep. agalactiae*	*E. coli*	*Staph. aureus*
< 200	26.1	6.5	-	2.2	-
200 – 400	56.7	20.0	23.3	16.7	0.8
> 400	70.0	16.7	13.3	16.7	-
	p≤0.001	p=0.202	p≤0.001	p≤0.05	p=0.566

found in milk with SCC of 200 000 to 400 000 cells/ml, and the highest CNS content was found in milk with more than 400 000 cells/ml.

Discussion

The most frequently-identified opportunistic pathogens known to cause clinical mastitis are *E. coli*, *S. aureus* (Olde Riekerink et al. 2008, Bradley et al. 2015) and *Streptococcus* species (Levison et al. 2016). In addition, coagulase-negative staphylococci have also been found to cause clinical mastitis (Taponen et al. 2016). CNS are increasingly being observed worldwide as the main etiological agent of this disease.

Regarding the impact of milk composition indicators, pathogen count increased with milk fat content, but decreased with increasing protein content (p≤0.01). Previous studies have found *Staphylococcus* spp. present in cow milk to depend on whether animals are

fed indoors or outdoors (Hagi et al. 2010), as well as on location and lactation period (Quigley et al. 2013). Our present findings confirm the presence of significant relationships between pathogen prevalence and cow lactation month ($p \leq 0.01$), SCC ($p \leq 0.001$) and milk protein content ($p \leq 0.01$). Hussain et al. (2012), Kline et al. (2018) and Youssif et al. (2021) report that both the age of the cows and the period of lactation appear to be important factors associated with the occurrence of mastitis (Kibebew et al. 2017, Vangroenweghe et al. 2020). Older cows are more susceptible to infections, most probably because of the wider or permanently partially-open teat canal as a results of frequent milking (Kibebew et al. 2017), also mammary epithelium of older cow has increased permeability, mainly because of the irreversible damage caused by previous inflammations (Król et al. 2013), meanwhile younger cows are much less likely to develop mastitis due to the reasons mentioned above. During transition period (between 3 weeks before and after parturition) dairy cows are at a higher risk to acquire diseases like mastitis also. Intramammary infections occurs more at parturition and first month of lactation (De Visscher et al. 2016). Our present findings indicate that among the composite milk samples with bacterial growth, CNS was the most commonly isolated bacterial group. Prevalence of pathogens was 42% lower in the milk of the first lactation cows than cows in their fourth or later lactation. Coagulase negative staphylococci were the most commonly identified in the youngest and oldest cows milk and accounted for 39.1% and 65.0% of the total counts. Our data also indicate a significant difference in *Enterococcus* spp. count between the first and the fourth and older lactations ($p \leq 0.05$). Duse et al. (2021) showed that *E. coli* was more common in peak and mid lactation, while *Staph. aureus* and *Trueperella pyogenes* were mainly present in early lactation. The authors showed that all pathogens except *E. coli* and *Strep. dysgalactiae* had a seasonal distribution. Our results showed that all pathogens except the *Enterococcus spp.* were found to increase with lactation number. In addition, during the first lactation, higher pathogen isolation rates were noted in the late lactation period (95.0%) than the early lactation period (38.5%; $p \leq 0.001$). During the third lactation period, higher CNN count was noted in the middle period of lactation ($p \leq 0.05$) while the highest count of *Enterococcus spp., S. agalactiae and E. coli* were fixed at the end of lactation. In the fourth and older lactation, compared to the early lactation period (months 1-4), higher *Enterococcus* spp. counts were found in the middle (5-8 months; $p \leq 0.05$) and later periods of lactation (nine months and above; $p \leq 0.01$). These findings contradict those of Zhang et al. (2016), who note a higher isolation rate in the early lactation period (60.18%) than in the late lactation period (50.95%; $p \leq 0.01$). The number of somatic cells in milk is a good indicator of the health of the herd. If milk production decreases, and its composition changes with an increase in the number of somatic cells and mastitis causing pathogens, it can be suspected that cows have latent mastitis. Our findings indicate that when the udder of cows is healthy and the milk SCC is less than 200 000 cells/ml, the presence of CNS and *E. coli* in milk is low. Kaczorek-Łukowska et al. (2021) found SCC level to be connected with specific pathogens: *Staph. aureus* and CNS were mainly isolated from samples with SCC from 200 000 to 2 000 000 cells/ml. The lowest CNS count was detected in milk with SCC values up to 200 000 cells/ml, and the highest counts in milk with SCC from 200 000 cells/ml to more than 400 000 cells/ml ($p<0.001$). Both *Staph. aureus* and *Strep. agalactiae* were observed in milk with SCC from 200 000 to 400 000 cells/ml ($p<0.001$). However, while Zhang et al. (2016) and Ágredo-Campos et al. (2023) also indicated a significant relationship between SCC and the prevalence of *E. coli*, their findings contradict our present results.

In our study the most common bacteria were the CNS. CNS and *Strep. agalactiae* counts increased with SCC, lactation number and lactation period. *E. coli* increased at the end of lactation period ($p \leq 0.05$). The *Enterococcus* spp**.** count in milk differed significantly between the first lactation and the fourth and older lactations ($p<0.05$). Also, the presence of pathogens was found to influence milk composition indicators: pathogen number increased with milk fat content but decreased with protein content ($p \leq 0.01$). In turn, Kester et al. (2015) observed a slight effect on fat and protein content.

Conclusions

Our results showed that among 1712 milk samples collected from lactating cows about 82% were infected with various pathogens. The most frequently isolated pathogen was coagulase-negative staphylococci (CNS) (46.7%), ahead of *S. agalactiae* (11.21%), and *E. coli* (10.28%).

Staphylococcus aureus was the most rarely isolated bacterial pathogen (0.23%). Coagulase-negative staphylococci and *S. agalactiae* tended to increase with increasing somatic cell count, lactation, and stage of lactation. In the older cows' milk are more bacteria, and lower count of somatic cells that fight off those bacteria, therefore older cows have a bigger chance of contracting an intra-mammary infection than low-age animals. *E. coli* increased at the end of lactation, and *Enterobacteria* spp. count increased from the 1st to the 4th and

older lactation cows. Analysis of the bacterial species as bovine subclinical mastitis agent would be actual for the development of mastitis control programs and to improve the health status of dairy herds.

References

Ágredo-Campos ÁS, Fernández-Silva JA, Ramírez-Vásquez NF (**2023**) Staphylococcus aureus, Escherichia coli, and Klebsiella spp. prevalence in bulk tank milk of Colombian herds and associated milking practices. Vet World 16: 869-881.

Antanaitis R, Juozaitiene V, Jonike V, Baumgartner W, Paulauskas A (**2021**) Milk Lactose as a Biomarker of Subclinical Mastitis in Dairy Cows. Animals (Basel) 11: 1736.

Bradley AJ, De Vliegher S, Green MJ, Larrosa P, Payne B, van de Leemput ES, Samson O, Valckenier D, Van Werven T, Waldeck HWF, White V, Goby L (**2015**) An investigation of the dynamics of intramammary infections acquired during the dry period on European dairy farms. J Dairy Sci 98: 6029-6047.

Breen JE, Green MJ, Bradley AJ (**2009**) Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci 92: 2551-2561.

Cheng J Qu W, Barkema HW, Nobrega D B, Gao J, Gang L, De Buck J, Kastelic JP, Sun H, Han B (**2019**) Antimicrobial resistance profiles of 5 common bovine mastitis pathogens in large Chinese dairy herds. J Dairy Sci 102: 2416-2426.

Cheng WN, Han SG (**2020**) Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments – A review. Asian-Australas J Anim Sci. 33: 1699-1713.

Cobirka M, Tancin V, Slama P (**2020**) Epidemiology and Classification of Mastitis. Animals (Basel) 10: 2212.

De Visscher A, Piepers S, Haesebrouck F, De Vliegher S (**2016**) Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health. J Dairy Sci 99: 6457-6469.

Duse A, Persson-Waller K, Pedersen K (**2021**) Microbial aetiology, antibiotic susceptibility and pathogen-specific risk factors for udder pathogens from clinical mastitis in dairy cows. Animals (Basel) 11: 2113.

Elmaghraby MM, El-Nahas AF, Fathala MM, Sahwan F, Tag EL-Dien MA (**2017**) Incidence of Clinical Mastitis and its Influence on Reproductive Performance of Dairy Cows. Alex J Vet Sci 54: 84-91.

Goli M, Ezzatpanah H, Ghavami M, Chamani M, Doosti A (**2012**) Prevalence assessment of Staphylococcus aureus and Streptococcus agalactiae by multiplex polymerase chain reaction (M-PCR) in bovine sub-clinical mastitis and their effect on somatic cell count (SCC) in Iranian dairy cows. Afr J Microbiol Res 6: 3005-10.

Hagi T, Kobayashi M, Nomura M (**2010**) Molecular-based analysis of changes in indigenous milk microflora during the grazing period. Biosci Biotechnol Biochem 74: 484-487.

Hussain R, Khan A, Javed MT, Rizvi F (**2012**) Possible risk factors associated with mastitis in indigenous cattle in Punjab, Pakistan. Pak Vet J 32: 605-608.

Jagielski T, Krukowski H, Bochniarz M, Piech T, Roeske K, Bakuła Z, Wlazło Ł, Woch P (**2019**) Prevalence of Prototheca spp. on dairy farms in Poland – a cross-country study. Microb Biotechnol 12: 556-566.

Kaczorek-Łukowska E, Małaczewska J, Wójcik R, Duk K, Blank A, Siwicki AK (**2021**) Streptococci as the new dominant aetiological factors of mastitis in dairy cows in north-eastern Poland: analysis of the results obtained in 2013-2019. Ir Vet J 74: 2.

Kayano M, Itoh M, Kusaba N, Hayashiguchi O, Kida K, Tanaka Y, Kawamoto K, Gröhn YT (**2018**) Associations of the first occurrence of pathogen-specific clinical mastitis with milk yield and milk composition in dairy cows. J Dairy Res 85: 309-316.

Kester HJ, Sorter DE, Hogan JS (**2015**) Activity and milk compositional changes following experimentally induced Streptococcus uberis bovine mastitis. J Dairy Sci 98: 999-1004.

Kibebew K (**2017**) Bovine Mastitis: A Review of Causes and Epidemiological Point of View. J Biol Agric Health 7: 1-14.

Kline KE, Flores S, Joyse F (**2018**) Factors affecting Somatic Cell Count in milk of dairy cows in Costa Rica. Int J Vet Sci Res 8: 1-8.

Król J, Brodziak A, Litwińczuk Z, Litwińczuk A (**2013**) Effect of age and stage of lactation on whey protein content in milk of cows of different breeds. Pol J Vet Sci 16: 395-397.

Law of the Republic of Lithuania on Animal Welfare and Protection No XI-2271 (**2012**) Official Gazette 'Valstybės žinios'. https://e-seimas.lrs.lt/portal/legalAct/lt/TAD/TAIS.434660

Levison LJ, Miller-Cushon EK, Tucker AL, Bergeron R, Leslie KE, Barkema KW, DeVries TJ (**2016**) Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. J Dairy Sci 99: 1341-1350.

Morales-Ubaldo AL, Rivero-Perez, N, Valladares-Carranza B, Velazquez-Ordoñez, V, Delgadillo-Ruiz L, Zaragoza-Bastida A (**2023**) Bovine mastitis, a worldwide impact disease: Prevalence, antimicrobial resistance, and viable alternative approaches. Vet Anim Sci 21: 100306.

Olde Riekerink RG, Barkema HW, Kelton DF, Scholl DT (**2008**) Incidence rate of clinical mastitis on Canadian dairy farms. J Dairy Sci 91: 1366-1377.

Petzer IM, Karzis, J, Donkin EF, Webb EC, Etter EM (**2017**) Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status. J Vet Res 84: e1-e10.

Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD (**2013**) The complex microbiota of raw milk. FEMS Microbiol Rev 37: 664-698.

Rifatbegović M, Nicholas RAJ, Mutevelić T, Hadžiomerović M, Maksimović Z (**2024**) Pathogens Associated with Bovine Mastitis: The Experience of Bosnia and Herzegovina. Vet Sci 11: 63.

Souza FN, Cunha AF, Rosa DL, Brito MA, Guimarães AS, Mendonça LC, Souza GN, Lage AP, Blagitz MG, Libera AM, Heinemann MB, Cerqueira MM (**2016**) Somatic cell count and mastitis pathogen detection in composite and single or duplicate quarter milk samples. Pesq Vet Bras 36: 811-818.

Sumon SMMR, Parvin MS, Ehsan MA, Islam MT (**2020**) Dynamics of somatic cell count and intramammary infection in lactating dairy cows. J Adv Vet Anim Res 7: 314-319.

Taponen S, Liski E, Heikkilä AM, Pyörälä S (**2017**) Factors associated with intramammary infection in dairy cows caused by coagulase-negative staphylococci, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalacti-

ae, Corynebacterium bovis, or Escherichia coli. J Dairy Sci 100: 493-503.

Vangroenweghe F, Duchateau L, Burvenich C (**2020**) Short communication: J-5 *Escherichia coli* vaccination does not influence severity of an Escherichia coli intramammary challenge in primiparous cows. J Dairy Sci 103: 6692-6697.

Watts LJ (**1988**) Etiological agents of bovine mastitis. Vet Microbiol 16: 41-66.

Youssif NH, Hafiz NM, Halawa MA, Saad MF (**2021**) Association of selected risk factors with bovine subclinical mastitis. Acta Vet Bras 15: 153-160.

Zhang Z, Li XP, Yang F, Luo JY, Wang XR, Liu LH, Li HS (**2016**) Influences of season, parity, lactation, udder area, milk yield, and clinical symptoms on intramammary infection in dairy cows. J Dairy Sci 99: 6484-93.