A study of bovine mastitis, milking procedures and management practices on 25 Estonian dairy herds
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Abstract

Background: Mastitis prevalence, milking procedures and management practices were investigated in 25 big dairy herds supplying milk to an Estonian dairy company. The aim of the study was to provide information for the company to be used in their new udder health improvement program to be set up after the completion of this study.

Methods: Quarter milk samples were collected from 3,166 cows for bacterial analysis and SCC (somatic cell counting). During the farm visit the veterinarian filled in a questionnaire about milking procedures and management practices with the help of farm managers. If the milk SCC of a cow or of a quarter exceeded 200,000/ml, the cow was defined as having mastitis.

Results: The percentage of cows having inflammation in one or more quarters measured by SCC (200,000/ml) was 52.7%. Corynebacterium bovis, Staphylococcus aureus and coagulase negative staphylococci were the most common bacterial isolates. Women as farm owners, and participating in the milking, were associated with lower mastitis prevalence on the farm. Peat bedding was associated with higher mastitis prevalence.

Conclusion: We demonstrated relatively high mastitis prevalence in this study. Contagious bacteria (eg. S. aureus, C. bovis, S. agalactiae and coagulase negative staphylococci) caused most of the infections. These infections are usually spread from cow to cow at milking if the milking hygiene is not good enough. The mastitis situation could be improved by improving milking procedures and hygiene.

Background
Milk production is the most important branch of Estonian agriculture. Estonian milk industry faced major changes after the country gained independence in 1991 with the dissolution of the Soviet Union. Farming systems based on sovchoses and kolchozes have disappeared and they have been replaced by family holdings and big farms owned by companies. The abolition of sovchoses and kol-
chooses has also resulted in the bankruptcy of many dairy plants. The ability of small farms to invest in farm improvement is much more limited than that of bigger farms, resulting in differences between management practices, equipment and even in the feeding of cows.

After the political changes described above, few studies of mastitis have been carried out on Estonian dairy herds. Tilga and Raid examined 2,420 milk samples during the years 1998–1991, and found that 5% of Estonian cows had clinical mastitis and 15–30% subclinical mastitis [1]. Their criteria for a mastitic cow was ≥500,000 cells/ml in a milk sample. Staphylococci were the most prevalent pathogen (34%) in their study. Klaassen et al. studied somatic cell counts (SCC) of 9,220 cow milk samples of four different herds [2]. In their study, 7.8% of the cows had over one million cells/ml. Aasmäe et al. examined quarter milk samples having more than 400,000 cells/ml [3]. Udder pathogens were isolated from about one half of quarter milk samples having more than 400,000 cells/ml had over one million cells/ml.

In their study, 7.8% of the cows belonged to 12 owners. Between November 1998 and March 1999, a dairy advisor and a veterinarian collected quarter milk samples from all cows producing more than five litres of milk per day on one farm visit. Milk samples were collected from 3,166 cows. During the farm visit, the veterinarian filled in a questionnaire about milking procedures and management practices with the help of the farm managers. The questions concerned the breed and age of the herds, mean annual milk production, milking procedures, milking units/milker, installation year and maintenance of milking machines, measurement of vacuum, milking claw size, type of cowshed, bedding type and manure handling.

Sampling
Sampling procedures and laboratory analyses were carried out as in a Finnish mastitis survey in 1995 [5,6]. Quarter milk samples were collected aseptically immediately before milking. The teat ends were cleaned with alcohol (70%) swabs and allowed to dry. The first few streams were discarded and the milk samples (about 5 ml) were collected in sterile 10 ml plastic tubes. Samples were immediately cooled and transported in cool bags to the Estonian National Veterinary Laboratories either in Tartu, Paide or Võru. Another non-aseptic quarter milk sample of 40 ml was taken in a 40 ml plastic tube for SCC counts from each quarter. The samples were cooled and transported to the laboratory of the Institute of Animal Husbandry at the Estonian Agricultural University in Tartu.

Analysis of milk samples
The SCC values of 12,328 quarter milk samples were measured by using the Fossomatic Milko Scan System 215 (Foss Electric, Hillerod, Denmark). A quarter was considered to have mastitis when the SCC was ≥200,000 [7,8]. Both subclinical and clinical cases are included in the results. To get a true average SCC of the farms, a value weighted by milk production was calculated from the analysed quarter milk samples by multiplying the average SCC of each cow by its milk production and then dividing the average results by the total milk production of each farm.

Microbiological analyses were carried out by streaking out 10 μl of milk with a sterile calibrated plastic loop on Trypticase Soy Agar plates (BBL, Cockeysville, MD, USA) containing 5% bovine blood. The plates were incubated at 37 ± 1°C for 48 hours. The plates were observed for bacterial growth after an incubation period of 24 and 48 hours. Bacterial species were identified according to Honkanen-Buzalski and Seuna [9].

Data analyses
The association of milking procedures and management practices with mastitis prevalence and the prevalence of bacterial pathogens (listed in table 1) was analysed statistically at herd level. The prevalence of mastitis and the prevalence of each bacteria species in the herds were used

Materials and methods
Study design
All farms (n = 25) producing milk for the dairy plant were selected for the study. The farms belonged to 12 owners. Between November 1998 and March 1999, a dairy advisor and a veterinarian collected quarter milk samples from all
as dependent variables and the data collected in the questionnaire were used as independent or categorical variables. Each variable was first analysed pairwise using t-test, the analysis of variance or correlation depending on the data structure. Their overall effect was further evaluated by analysing parameters using stepwise regression. Only the significant findings of regression analysis are reported. The statistical analysis was carried out with Statistix software (Analytical software, Tallahassee, USA).

Results

Somatic cell counts

The distribution of healthy and mastitic udder quarters with or without bacteria is shown in Figure 1. The proportion of quarters having SCC ≥200,000/ml was 25.3% (95% confidence interval 22.3 to 28.3) (Fig. 1). The percentage of cows having mastitis in one or more quarters, as measured by SCC, was 52.7% (95% confidence interval, 47.9 to 57.6). The average SCC calculated from quarter milk samples weighted by milk production was 370,120 cells/ml (95% confidence interval 313,970 to 426,270).

Mastitis pathogens

The distribution of bacterial findings in 11,640 quarter milk samples, the proportions of bacterial isolates and the corresponding SCC are shown in Table 1. Samples with mixed cultures were excluded from these figures as these quarters cannot be categorised either as healthy or mastitic. Corynebacterium bovis was isolated in 14.6% of the samples and was the most common bacteria isolated (47.3% of isolates). Staphylococcus aureus was isolated from 6.5% of the samples and coagulase negative staphylococci from 4.9%. Streptococci were less frequent isolates.

Herd, milk production and management procedures

The cow breeds in the investigated 25 herds were Estonian Red, and Estonian Holstein. Fourteen herds included both breeds, two herds contained only Estonian Red, and nine herds only Estonian Holstein. The average herd size was 164.4 cows, ranging from 15 to 463. The average age of the animals was 5.3 years. All herds are catalogued in the Estonian Animal Recording System. According to the data, the average production/cow was 5,520 kg/year. The latest bulk milk SCC value of the herds before the farm visit averaged 304,560 cells/ml (95% confidence interval 250,400 to 358,700), ranging from 69,000 to 730,000 cells/ml.

The animals were kept in tie-stall housing (88% of animals) at 22 farms, and only three herds had loose housing. Only two herds were not pasturing. The bedding material was sawdust in 32%, straw in 16%, cut straw in 4%, peat in 8% and a combination of the previously mentioned materials in 40% of the herds. All herds had solid manure removal. Peat bedding was associated with higher mastitis prevalence (p < 0.01).

The farms had seven different milking machine types. The age of the milking machines varied from one to 22 years. The milk claws were smaller than 150 ml in 16% of the milking machines. All milking machines had been serviced after 1996 and 80% of the milking machines had been vacuum tested during the previous year. The average number of milking units/milker was 3.6, with a minimum of two and maximum of 16. The average number of cows/milker was 64.2, with a minimum of 15 and maximum of 155. Only seven farms used teat-dipping. Women as farm owners, and participating in the milking, were associated with lower mastitis prevalence at the farms (p < 0.05).

Table 1: Bacterial findings during the survey.

| Bacterial isolate                   | No of samples N = 11,640 | % of all samples | % of isolates N = 3,581 | SCC ×10³/ml (SD) |
|------------------------------------|--------------------------|------------------|-------------------------|------------------|
| Streptococcus agalactiae           | 110                      | 0.9              | 3.1                     | 3,263 (5,596)    |
| Streptococcus dysgalactiae         | 68                       | 0.6              | 1.9                     | 2,576 (4,041)    |
| Streptococcus uberis               | 86                       | 0.7              | 2.4                     | 2,431 (4,452)    |
| Other streptococci, Enterococci    | 208                      | 1.8              | 5.8                     | 1,706 (3,100)    |
| Staphylococcus aureus              | 752                      | 6.5              | 21.0                    | 1,026 (2,070)    |
| Coagulase-negative staphylococci   | 568                      | 4.9              | 15.8                    | 508 (1,765)      |
| Corynebacterium bovis              | 1,694                    | 14.5             | 47.3                    | 303 (956)        |
| Coliforms                          | 20                       | 0.2              | 0.6                     | 5,401 (5,423)    |
| Actinomyces spp.                   | 7                        | 0.1              | 0.2                     | 12,579 (9,262)   |
| Other bacteria                     | 68                       | 0.6              | 1.9                     | 565 (2,165)      |
| No growth                          | 8,059                    | 69.2             | 221 (961)               |

The figures in the first two columns are the numbers and percentages of all quarter milk samples. The third column represents percentages of bacterial isolates. Mean SCC (SD) for each group are also presented.
Other milking practices and some management procedures are described in Table 2.

None of the above-mentioned properties or management procedures were statistically associated with the presence of certain bacteria.

Discussion

The farms in this study did not represent the usual type of Estonian farms, and the results should be compared only with farming in larger herds. The mean annual milk production (5,701 kg) in the herds of this study was somewhat higher than the average production of cows in Estonia (4,766 kg). The mean age of the cows was seven months lower than the average age of Estonian cows. Herd sizes were bigger than the average in Estonia, where about 50% of the herds have less than 10 cows [10]. In this study, the mean size of herds was 164.4 cows and only three herds had less than 20 cows.

It is usually difficult to compare results of surveillance studies because of differences in the sample selection and the criteria of mastitis. This study was carried out in a similar fashion as mastitis surveys in Finland in which a threshold level of 300,000 cells/ml were used for mastitis as suggested by Klastrup and Schmidt Madsen [6,11,12]. New data indicate, however, that the SCC of a cow that is not infected with mastitis pathogens is usually < 200,000 cells/ml and therefore this threshold level was used in this study [7,8]. To compare these results with the Finnish studies, we calculated mastitis prevalence also with a threshold level of 300,000 cells/ml. Thus the percentage of cows having mastitis in one or more quarters was 43.5% (95% confidence interval, 39.3 to 47.3), which was slightly higher than in Finland where the corresponding figure was 38% (95% confidence interval 35.1 to 40.5) in 1995 and 31% (95% confidence interval 28.4 to 33.1) in 2001 [11].

The mean SCC value as calculated from individual quarter milk samples appeared higher than that measured from the bulk milk (p = 0.07). The reason for this may be the fact that milk from cows suffering from acute clinical mastitis may have been treated with antibiotics and the milk discarded. Therefore the SCC of tank milk can give too optimistic a figure of the herd mastitis situation if it is the only measurement criteria. In the study conducted by Klaassen et al study, only 7.8% exceeded one million cells/ml (cow sample), which could be explained by the fact that in their material, milk samples were only collected from milk supplied to dairy plants [2]. In this study, 20.7% of cows had more than one million cells/ml (the average of quarter milk samples).

C. bovis was the most common bacterial finding in the study, which is in line with the Finnish study by Pitkälä et al [11]. Honkanen-Buzalski et al. showed that C. bovis infection levels can differ considerably between herds, and that C. bovis infections are most common in herds not using teat dipping [13]. In this study, only 7 out of 25 herds used teat dipping, which may explain the high prevalence of C. Bovis. However, statistically significant differences were not detected. In a study conducted by Aasmäe et al., C. bovis was only found in 0.6% of the samples, but milk samples having less than 300,000 cells/ml were not investigated [3]. As C. bovis infections are normally mild, they may remain undetected [14]. The mean SCC of quarters with C. bovis was 303,000/ml. However, only 27.4% of the quarters with C. bovis infection had SCC ≥200,000/ml.

In quarters infected with major udder pathogens, the
mean SCC was ≥ 1,000,000/ml. Staphylococcal infections, *S. aureus* and CNS, were as common in this study as in the study of Aasmäe et al. [3].

Contagious bacteria (eg. *S. aureus*, *C. bovis* and *S. agalactiae*) caused most of the infections in this study. These infections are usually spread from cow to cow at milking if the milking hygiene is not good enough. Therefore it is likely that the mastitis situation could be improved by improving milking procedures and hygiene. It is known that milking procedures and practices followed by milkers strongly influence the prevalence of mastitis in herds as well as the overall milk quality [8,15]. In some of the herds, a milker used more than three milking units simultaneously, which can further impair the milking routine. However, milking procedures such as teat cleaning, udder preparation before milking, and overmilking were not observed in this study.

The farms in this study had clearly defined, separate work tasks between personnel. Typically, it was only milkers who did the milking. Each milker had his/her own group of about 60–65 cows. The employees’ job descriptions were of the rigid kind and did not contribute to the prevention of mastitis. In the few cases where women owners participated in the milking, the prevalence of mastitis was lowest. This finding could be in line with the finding of Barnouin et al., according to which a herdsman precise in his techniques was associated with the herd having low SCC [16]. Owners probably are more motivated to maintain precise milking techniques than hired personnel and therefore a clearer motivation-based approach for the responsibilities of employees and supervisors would improve the mastitis situation.

The finding that peat was associated with higher mastitis prevalence could be due to difficulties in keeping animals clean. This association has not been observed in some other studies. Hovinen et al. observed that bedding material (peat, sawdust or straw) on the teat was cleaned almost completely in automatic milking herds, and Peltola found out that peat litter had no effect on the state of health of the animals or on the quality of the milk [17,18].

There were also other deficiencies in housing conditions and milking machines, which should be corrected. In many herds, for instance, the stalls were too short for the cows of today. The milk claws were too small (less than 150 ml) in some herds [19]. Even though statistical analysis did not point out specific risk factors, such as milking claws being too small or the infrequent use of teat dipping, it can be assumed that by correcting these deficiencies, udder health and milk quality could also be improved.

**Conclusion**

Relatively high mastitis prevalence was revealed in this study. *S. aureus*, *C. bovis*, *S. agalactiae* and coagulase negative staphylococci caused most of the infections. These bacteria are easily spread from cow to cow at milking. Interestingly, peat was found to associate with higher mastitis prevalence. These results suggest that the mastitis situation could be improved by improving milking procedures and hygiene. Furthermore, the farms had clearly defined, separate work tasks between personnel. Clearer motivation-based approach for the responsibilities of employees and supervisors would contribute to the prevention of mastitis.

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors’ contributions**

LH took part in all aspects of investigation including planning, microbiological laboratory work and drafting of the manuscript. She carried out the sampling. TH-B participated in planning the study and drafting the manuscript. IS participated in planning and performing microbiological laboratory work. AO coordinated somatic cell counting in laboratory. VM carried out the data analyses and participated in drafting the manuscript.

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**References**

1. Tõiga V, Raid H: Stafülokokkilisest mastiidiidest veistel. Bovine staphylococcal mastitis. The Estonian veterinary review 1994, 4:18-19, 70.
2. Klaassen M, Peterson K, Kihu J: Udara tervisliku seisundi hindamine soomastiliste rakkude arvu (SRA) alusel 1 cm3 piimas. Evaluation of the health status of the udder on basis of somatic cell count (SCC) in 1 cm3 of milk. The Estonian veterinary review 1995, 5:196-199.
3. Aasmäe B, Kalmus P, Ööpik T, Klaassen E: Mastiidipatogeenide tundlikkus antibiootikumide suhtes. Antimicrobial susceptibility of bovine mastitis pathogens. The Estonian veterinary review 2000, 1:9-11.
4. Mölder M, Meijerei Laeva: financial manager, personal communication. Tartu. 1999.
5. Honkanen-Buzalski T: Sampling technique, transportation and history. In The bovine udder and mastitis Edited by: Sandholm M, Honkanen-Buzalski T, Kaarinen L, Pyörälä S. Gummerus kirjapaino Oy, Jyväskyla, Finland; 1995:112.
6. Myllä M, Asplund K, Brofeldt E, Hirvela-Koski V, Honkanen-Buzalski T, Junttila J, Kulkas L, Myllykangas O, Niskanen M, Saloniemi H, Sandholm M, Saranpää T: Bovine mastitis in Finland in 1998 and 1995 – Changes in prevalence and antimicrobial resistance. Acta vet Scand 1998, 39:119-126.
7. Ruegg PL, Reinemann DJ: Milk quality and mastitis tests. Bovine Pract 2003, 36:41-54.
8. Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L, Gonzales RN: Monitoring udder health and milk quality using somatic cell counts. Vet Res 2003, 34:579-596.
9. Honkanen-Buzalski T, Seuna E: Isolation and identification of pathogens from milk. In The bovine udder and mastitis Edited by:
Sandholm M, Honkanen-Buzalski T, Kaartinen L, Pyörälä S. Gummerus kirjapaino Oy, Jyväskylä, Finland; 1995:121-141.

10. Agricultural register and information centre: Results of animal recording in Estonia 1998. Estonia 1999. 5, 14, 15, 23, 29, 34

11. Pilkala A, Haveri M, Pyörälä S, Myllys V, Honkanen-Buzalski T: Bovine Mastitis in Finland 2001. J Dairy Sci 2004, 87:2433-2441.

12. Klastrup O, Schmidt Madsen P: Nordiske rekomendationer vedrørende mastitundesøgelser af kiertelprøver. Nord Vet Med 1974, 26:197-204.

13. Honkanen-Buzalski T, Griffin T, Dodd F: Observations on Corynebacterium bovis infection of the bovine mammary gland. I. Natural infection. J Dairy Res 1984, 51:371-378.

14. Honkanen-Buzalski T, Bramley AJ: Observations on Corynebacterium bovis infection of the bovine mammary gland. II. Experimental infection. J Dairy Res 1984, 51:379-385.

15. Peeler EJ, Green MJ, Fitzpatrick JL, Morgan KL, Green LE: Risk factors associated with clinical mastitis in low somatic cell count british dairy herds. J Dairy Sci 2000, 83:2464-2472.

16. Barnouin J, Chassagne M, Bazin S, Boichard D: Management paractices from questionnaire surveys in herds with very low somatic cell score through a national mastitis program in France. J Dairy Sci 2004, 87:3989-3999.

17. Hovinen M, Aista AM, Pyorala S: Visual detection of technical success and effectiveness of teat cleaning in two automatic milking systems. J Dairy Sci 2005, 88:3354-3362.

18. Peltola I: Use of peat as litter for milking cows. In proceedings of Odour prevention and control of organic sludge and livestock farming Seminar: 15–19 April 1985, Silsoe UK Editted by: Nielson VC, Voorburg JH, L’Hermite P. London: Elsevier Applied Science Publishers; 1986:181-187.

19. Manninen E: Effect of milking and milking machine on udder health. In the bovine udder and mastitis Jyväskylä, Finland, Gummerus kirjapaino Oy; 1995:236.