Short communication:

The prevalence of some mastitis pathogens in bulk milk of Dutch dairy goats and the relationship with bulk milk somatic cell count and bulk milk standard plate count.

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Abstract

The aim of this study was to assess the correlation of bulk milk somatic cell count (BMSCC) with bulk milk standard plate count (BMSPC) and with the number of coliform bacteria, coagulase negative staphylococci (CNS) and *S. aureus*. We also described the prevalence of these pathogens in bulk milk of Dutch dairy goat farms. In total 53 dairy goat farms participated in this study. Bulk milk was collected 3 times with an interval of two weeks during the months January, February and March and was examined on BMSCC, BMSPC, bulk milk coliform count (BMC), bulk milk coagulase negative staphylococci count (BMCNS), bulk milk manitol fermenting staphylococci count (BMMFS) and on the presence of *S. aureus*. No correlation was found between BMSCC and BMSPC. Significant factors affecting BMSCC were BMCNS (correlation coefficient 0.16) and BMMFS (correlation coefficient 0.08). The amount of *S. aureus* positive milk samples (0,1,2 or 3 out of 3) did not affect the mean BMSCC. BMSPC was determined for 20% by BMMFS and for 13% by BMCNS. Coliforms do not seem to affect BMSCC and BMSPC. *S. aureus* was present in 53.5% of 159 milk samples. On 85% of all the participating dairy goat farms *S. aureus* was found in 1 or more milk samples. On 20.8% of the participating farms *S. aureus* was found in all milk samples. All milk samples contained CNS and 86.8 % of the samples contained coliforms. This study shows that mastitis pathogens are responsible for some of the variation in BMSCC and BMSPC, suggesting that udder health programs can positively influence the BMSCC and BMSPC. Therefore both BMSCC and BMSPC can be useful tools in evaluating udder health status of dairy goat herds.

Keywords: Dairy goat, bulk milk, somatic cell count, standard plate count, mastitis pathogens

Introduction

To prevent and control mastitis on dairy goat farms it is important to gain knowledge of the prevalence of mastitis pathogens and their association with bulk milk somatic cell count (BMSCC) and bulk milk standard plate count (BMSPC).

The total bacterial count is commonly used to evaluate the bacteriological quality of bulk milk (Koop et al., 2009). Although bacteriological quality is affected the most by clean milking systems, hygienic transport and rapid refrigeration, a high prevalence of intramammary infections will contribute to an increase in BMSPC (Contreras et al., 2003).

The correlation between BMSPC and the presence of different mastitis pathogens in bulk milk has been described in cows (Jayarao et al., 2004) and in dairy goats (Foschino et al., 2002), but has not been studied in The Netherlands yet.

The BMSCC is affected by several factors, such as seasonal variation (Koop et al., 2009) and hygiene sanitary management conditions (Delgado-Pertiñez et al., 2003). But mastitis related factors such as treating mastitis animals instead of culling also affect BMSCC significantly (Koop et al., 2009).
In cows a correlation was found between different mastitis pathogens and BMSCC (Jayarao et al., 2004, Olde Riekerink et al., 2006), while in goatherds no correlation between BMSCC and mastitis pathogens was found (Foschino et al., 2002). No research on this correlation has yet been done in The Netherlands.

There has been considerable controversy on the relationship between BMSCC and BMSPC. The studies of Ying et al. (2002) and Delgado-Pertiñez et al. (2003) showed no significant correlation between the BMSCC and BMSPC in bulk milk of dairy goats. The study of Koop et al. (2009) revealed a positive correlation between BMSCC and bulk milk total bacterial count (BMTBC) in goat milk. Zeng et al. (1995) describes a positive relation between SCC and SPC in individual goats. A positive relationship between BMSCC and BMSPC was also found in the bulk milk of dairy sheep (Gonzalo et al., 2006) and in the bulk milk of cows (Jayarao et al., 2004).

The purpose of this paper was to describe the correlation between BMSCC, BMSPC, coliforms, coagulase negative staphylococci and *S. aureus*, and to describe the prevalence of these pathogens in bulk milk of Dutch dairy goats.

Materials and methods

Farms and sample collection

In total, 53 goat farms were selected to participate in this research, including 48 Dutch dairy goat farms and 5 Belgium goat farms. All participating farms were randomly selected from a large goat milk processing company in The Netherlands.

Bulk milk samples were collected three times, with an interval of two weeks during the months January, February and March. Four farms had an interval of three weeks and 15 farms had an interval of four weeks between the second and third milk sample.

Samples were collected by milk lorry drivers, transported on melting ice, stored at below 5 ºC and processed for bacteriological analysis within 24 h. A second sample was taken and analyzed for bulk milk somatic cell count (BMSCC) by use of the Fossomatic 5000 apparatus (Foss, Hillerød, Denmark).

Bacteriological procedures

All bacteriological procedures were performed according to the protocol for bulk milk of The National Mastitis Council (Anonymous 1990). To determine the total bacterial growth, plate count agar (Oxiod, Hampshire, United Kingdom) was used. An amount of 0.1 ml of the dilutions 10^{-2}, 10^{-3} and 10^{-4} was evenly spread with a sterile spatula on the plate count agar (PCA). All dilutions were made using peptone physiological salt solution. After three days of incubation at 30 ºC the number of colonies was determined (colony forming units (cfu)/ml milk).

The amount of coliform bacteria was determined using MacConkey agar (Oxiod, Hampshire, United Kingdom). An amount of 0.1 ml of the dilutions 0, 10^{-1} and 10^{-2} was used on the agar. After 24 h of incubation at 37 ºC the number of coliform colonies (cfu/ml milk) was determined.

To determine the amount of *Staphylococcus spp* mannitol salt agar (Oxiod, Hampshire, United Kingdom) was used. An amount of 0.1 ml of the dilutions 0, 10^{-1} and 10^{-2} was spread on the plates. After 24 h of incubation at 37 ºC the colonies on the mannitol salt agar plates were defined as mannitol fermenting (MF) or not mannitol fermenting (NMF). After 48 h of incubation the number of MF and NMF colonies were counted (cfu) and converted to cfu/ml milk.

After 24 h three MF colonies of each milk sample were transferred to separate tryptic soy agar plates (Oxiod, Hampshire, United Kingdom) to obtain a pure culture. These tryptic soy agar plates were incubated for 24 h at 37 ºC. After 24 hours of incubation one colony from each plate was subjected to a catalase test by adding a drop of hydrogen peroxide (3%) to the separated colony. When oxygen was released the colony was positive on catalase enzymes. Most cytochrome-containing aerobic and facultative anaerobic bacteria, including staphylococci, are catalase positive. *Streptococcus* and *Enterococcus* species are exceptions and are catalase negative (Health Protection Agency, 2007).

Another colony of the same plate was subjected to a tube coagulase test. This colony was transferred to 0.5 ml rabbit coagulase plasma (Becton, Dickinson and company, Sparks, USA) and defined positive or negative after 3-4 hours incubation at 37 ºC. Some colonies need some more time to grow so definitive results can be seen at 24 hours of incubation. Coagulase positive colonies were defined as *S. aureus*. Coagulase positive tests were recorded as 1, 2 or 3 colonies positive and a milk sample was considered positive or negative on *S. aureus* regardless of the number of positive colonies. For each farm the number of positive milk samples was recorded.

Three NMF colonies of 5 randomly selected milk samples from different farms were also transferred to TSA plates and subjected to a catalase and coagulase test to confirm that NMF colonies are coagulase negative staphylococci (CNS).

With each set of samples a positive control was made of the MSA and MAC agar plates with pure cultures of *S. aureus* and *E. coli.* With each set a positive and negative control of the catalase test and tube coagulase test was also obtained with these cultures.

Statistical analysis

Descriptive statistics

Data was collected in Microsoft Excel 2003 and all statistical analyses were performed using SPSS for windows version 16.0 (SPSS Inc., 2007, Chicago, Illinois).

Bulk milk somatic cell count (BMSCC), bulk milk standard plate count (BMSPC), bulk milk coliform count (BMC), bulk milk manitol fermenting colony count (BMMF) and bulk milk not manitol fermenting colony count (BMNMF) were normalized by 10log transformation and measurements of the 3 bulk milk samples of each farm were averaged. Averaged variables are listed as mean-BMSCC, mean-BMSPC, mean-BMC, mean-BMMF and mean-BMNMF. Mean values and standard deviations of mean-BMSCC, mean-BMSPC, mean-BMC, mean-BMMF and mean-BMNMF were calculated and the Kolmogorov-Smirnov Test was used to test if these variables were normally distributed.

The influence of the different pathogens on the mean-BMSPC is determined with a mixed model. This model shows the percentage of mean-BMSPC that is determined by the different pathogens.

Correlations

To examine if BMSPC, BMC, BMMF and BMNMF affect the BMSCC, a linear regression model was made. The effect of the different sample rounds on the BMSCC was examined by a paired T-test.

The effect of the different sample rounds on the BMSPC is examined by a paired T-test.

The effect of the date of sampling on the BMSCC, BMSPC, BMC, BMMF and BMNMF is determined by a linear regression model.

We performed an ANOVA to determine the effect of the amount of *S. aureus* positive milk samples (0, 1, 2 or 3 out of 3) on the mean-BMSCC and mean-BMSPC.

In all tests, $P < 0.05$ was considered significant.

Results and discussion

Bacteriological analysis

The colonies found on the MSA plate without manitol fermentation (NMF) of 5 random milk samples were all tested catalase positive and coagulase negative and thus NMF colonies were determined as coagulase negative staphylococci (CNS) (Health Protection Agency. 2007, Chapman, 1945).

Manitol fermenting colonies (MF) are more difficult to determine, these colonies were suspected to be colonies of *S. aureus*, but only 34.2 % of the tested colonies were coagulase positive and thus confirmed as *S. aureus*. Bacterial organisms other than staphylococci are inhibited on the MSA plate, except for micrococci which have a different aspect, and because all the MF colonies tested catalase positive, it is likely that the MF colonies belong to the staphylococcus spp. (Health Protection Agency. 2007, Chapman, 1945).

It is difficult to determine the exact percentage of MF colonies that is *S. aureus* and the percentage that is CNS. The determination of the amount of CNS in this research is therefore limited to the count of NMF colonies, which means that the amount of CNS in the researched milk samples is underestimated.

The bulk milk bacterial count of non manitol fermenting bacteria (BMNMF) will be reported as bulk milk CNS count (BMCNS). The bulk milk bacterial count of manitol fermenting bacteria (BMMF) will be reported as bulk milk manitol fermenting staphylococci count (BMMFS).

Descriptive statistics

When comparing our results with the results of bulk milk quality researches in other European countries we can see that the overall mean value of standard plate count (mean-BMSPC) is lower and the overall mean value of somatic cell count (mean-BMSCC) is higher in The Netherlands than in other countries (Table 1).

Furthermore the overall mean value of coliform bacteria in this research (mean-BMC) is lower and the overall mean value of CNS is comparable with the results of Foschino et al. (2002) in Italy (Table 1).

The Kolmogorov-Smirnov Test showed that all log transformed variables were normally distributed. Table 2 shows the logarithmic means, ranges and standard deviations of all variables. Where Foschino et al. (2002) found a *S. aureus* prevalence of 43% in 60 milk samples, and Muehlherr et al. (2003) found a prevalence of 31.7% in 344 milk samples, we found a prevalence of 53.5% in 159 milk samples. On 85% of all the participating dairy goat farms in this research *S. aureus* was found in 1 or more milk samples. On 20.8% of the participating farms *S. aureus* was found in all milk samples. All milk samples contained CNS and 86.8 % of the samples contained Coliform bacteria.

Table 1. Comparison of the mean bulk milk somatic cell count (mean-BMSCC), mean bulk milk standard plate count (mean-BMSPC), mean bulk milk coliform count (mean-BMC), mean bulk milk manitol fermenting *Staphylococci* count (mean-BMMFS) and mean bulk milk coagulase negative *Staphylococci* count (mean-BMCNS) in different researches in different European countries.

		Netherlands This study (year 2009)	**Italy, bergamo region** Foschino et al., 2002	**Switserland** Muehlherr et al., 2003
mean-BMSCC	(cells / ml milk)	1.5×10^6	9.9×10^5	_
mean-BMSPC	(cfu / ml milk)	3.3×10^4	5.0×10^4	4.9×10^4
mean-BMC	(cfu / ml milk)	40.8	9.1×10^2	_
mean-BMMFS	(cfu / ml milk)	3.5×10^3	_	_
mean-BMCNS	(cfu / ml milk)	1.9×10^3	1.3×10^3	_

Table 2. Descriptive statistics of the 10log bulk milk mean somatic cell count (mean-BMSCC), 10log bulk milk mean standard plate count (mean-BMSPC), 10log bulk milk mean Coliform count (mean-BMC), 10log bulk milk mean mannitol fermenting *Staphylococci* count (mean-BMMFS) and 10log bulk milk mean Coagulase Negative *Staphylococci* count (mean-BMCNS)

	N (farms)	Mean	SD[1]	Minimum	Maximum
mean-BMSCC	53	6.17	0.13	5.92	6.53
mean-BMSPC	53	4.55	0.43	3.85	5.56
mean-BMC	53	1.61	0.92	0.00	3.82
mean-BMMFS	53	3.55	0.44	2.74	4.66
mean-BMCNS	53	3.29	0.48	2.10	4.11

[1]SD = standard deviation.

Correlation between BMSCC and BMSPC

No significant correlation could be found between BMSCC and BMSPC (Table 3). This observation corresponds to the findings of Ying et al. (2002). Delgado-Pertiñez et al. (2003) could not find a relationship between BMSCC and bulk milk total bacterial count (BMTBC). Controversial to the research of Delgado-Pertiñez et al. (2003), Koop et al. (2009) found a positive correlation between BMSCC and BMTBC. A positive correlation between SCC and SPC was also found in individual goats by Zeng et al. (1995) ($P<0.001$).

In cows a relationship between BMSCC and BMSPC was found by Jayarao et al. (2004). Jayarao et al. (2004) showed that bulk milk with a low (<5000 cfu/mL) standard plate count also had a significantly low level of mean bulk tank somatic cell count (<200,000 cells/mL). A positive correlation between BMSCC and BMSPC was also found in sheep (Gonzalo et al., 2006).

As these different researches show, there is still controversy about the relationship between BMSCC and the amount of bacteria in the bulk milk of goats. An explanation could be that various factors influence BMSCC and bulk milk bacterial count. Factors like seasonal variance (Koop et al., 2009), milk fever prevalence (Koop et al., 2009) and hygiene sanitary management conditions (Delgado-Pertiñez et al., 2003) affect the BMSCC. The amount of bacteria in bulk milk is affected the most by clean milking systems, hygienic transport and rapid refrigeration (Contreras et al., 2003), but seasonal variance also affects the amount of bacteria in bulk milk (Koop et al., 2009). The effect of these factors can be different for each research, since most of these researches are done in different countries.

As there is a seasonal variance the length of sample period can also be an explanation for the different outcomes of the researches. Ying et al. (2002) had a sample period of 2 months, Delgado-Pertiñez et al. (2003) collected milk samples during 9 months and Koop et al. (2009) had a sample period of 3 years. The sample period of our research was 7 weeks.

The number of participating farms and the number of analyzed milk samples per farm also differ between these researches. This could also contribute to the different outcomes.

Correlations between BMSPC and mastitis pathogens

The percentage of BMSPC determined by BMCNS, BMMFS and BMC is listed in table 4.

Table 3. Correlation coefficients (R) for 10log bulk milk mean somatic cell count (mean-BMSCC), 10log bulk milk mean standard plate count (mean-BMSPC), 10log bulk milk mean Coliform count (mean-BMC), 10log bulk milk mean mannitol fermenting *Staphylococci* count (mean-BMMFS) and 10log bulk milk mean Coagulase Negative *Staphylococci* count (mean-BMCNS)

	N (farms)	mean-BMSCC
mean-BMSCC	53	1.00
mean-BMSPC	53	0.12
mean-BMC	53	0.11
mean-BMMFS	53	0.28*
mean-BMCNS	53	0.40**

Statistic significance: * P<0,05; ** P<0,01; values without asterisks are not significant (P>0,05)

Table 4. Percentage of bulk tank standard plate count (BMSPC) that is determined by bulk milk Coliform count (BTC), bulk milk manitol fermenting *Staphylococci* count (BMMFS) and bulk milk CNS count (BMCNS)

	N (farms)	Percentage of BMSPC
BMC	53	0.8
BMMFS	53	19.7
BMCNS	53	12.8
total percentage	53	33.3

The bacteriological methods used in this research does have some restrictions, meaning that the used methods are not sufficient for determining the exact amount of CNS and *S. aureus* in bulk milk. It is unclear what percentage of BMMFS is *S. aureus* and what percentage is CNS.

What we can say is that BMSPC is determined for more than 32% by staphylococcus spp. and that coliform bacteria do not seem to determine the BMSPC. Earlier research showed that subclinical mastitis of dairy goats is caused in 79% by staphylococcus spp., of which 8% by *S. aureus* (Bergonier et al., 2003). Concluding that staphylococci are important organisms involved in dairy goat mastitis.

The amount of *S. aureus* positive milk samples (1, 2 or 3 out of 3) on each farm was not correlated with the BMSPC in our study, indicating that the presence of *S. aureus* is not affecting the BMSPC. The presence of *S. aureus* was determined by testing three manitol fermenting colonies on coagulase enzymes, more colonies should have been tested to give more certainty about the presence of *S. aureus* in a bulk milk sample.

Controversial to this research, the study of Foschino et al. (2002) revealed that the amount of coliforms in bulk milk of dairy goats is significantly correlated with the BMSPC (correlation coefficient 0.56).

The study of Foschino et al. (2002) also showed a significant correlation between coagulase positive staphylococci and BMSPC. BMCNS did not have a significant correlation with BMSPC in this study.

In cows Jayarao et al. (2004) found a significant correlation between BTSPC and CNS (correlation coefficient 0.57). BTSPC and coliform count were also correlated but with a lower correlation coefficient (0.18).

Correlations between BMSCC and mastitis pathogens

A positive significant correlation was found between BMCNS and BMSCC, and between BMMFS and BMSCC (Table 3). Although it is not possible to determine the precise correlation between *S. aureus* and BMSCC and between CNS and BMSCC, we can say that the amount of staphylococci in bulk milk affect the BMSCC. In dairy goat milk the study of Foschino et al. (2002) revealed no correlation between BMSCC and bacterial counts. The correlation between BMSCC and BMMFS is more or less comparable with the findings of Jayarao et al. (2004) in cows, given that the bulk milk count of manitol fermenting staphylococci consists of CNS and *S. aureus*. Jayarao et al. (2004) showed that *S. aureus* was significantly associated with an increased bulk milk somatic cell count.

The study of Olde Riekerink et al. (2006) showed a positive correlation between BMSCC and the amount of *S. aureus* positive milk samples in cows. In our study the amount of *S. aureus* positive milk samples (1, 2 or 3 out of 3) on each farm was not correlated with the BMSCC, indicating that the presence of *S. aureus* does not affect the BMSCC.

No significant correlation was found between BMSCC and BMC (Table 3). In bulk milk of cows a positive correlation between BMSCC and BMC was found by Rysanek et al. (2005).

Effect of the date and sample round

The number of the sample round has a significant effect on the BMSCC. The BMSCC decreases with each sample round. Sample round also had a significant effect on BMSPC, the amount of BMSPC increased with each sample round. When determining the effect of the date on BMSCC and BMSPC a little fluctuation is found. This could be explained by a normal fluctuation of the BMSCC and BMSPC during the year (Delgado-Pertiñez et al., 2003, Koop et al., 2009). Delgado-Pertiñez et al. (2003) showed that the highest bacterial counts in bulk milk samples of dairy goats are found in January and April.

No correlation is found between sample round and BMMFS, BMCNS and BMC and the date did not seem to influence these variables.

Conclusions

Although bulk milk somatic cell count (BMSCC) and bulk milk standard plate count (BMSPC) were not correlated in this study, both BMSCC and BMSPC are affected by mastitis pathogens.

Of the different pathogens examined in this study BMSCC is influenced the most by coagulase negative staphylococci.

Both BMCNS and BMMFS were positively correlated with BMSCC, indicating that staphylococci in bulk milk are responsible for an increase in bulk milk somatic cell count.

Although the correlation coefficients are not high and BMSCC is affected by more factors such as seasonal variation (Koop et al., 2009) and hygiene-sanitary management conditions (Delgado-pertiñez et al., 2003), it is likely that programs to improve udder health can contribute to a decrease in BMSCC.

BMSPC is determined for 32% by coagulase negative staphylococci and *S. aureus*. Coliforms, which are in goat milk more part of the hygienic aspect than of mastitis origin, do not have a significant effect on BMSCC and BMSPC.

This study shows that Staphylococci, which are the mean cause of subclinical mastitis in goats (Bergonier et al., 2003), play an interesting role in affecting the BMSCC and BMSPC.

In conclusion, both BMSCC and BMSPC can be valuable tools for evaluating and controlling udder health status of dairy goat herds in The Netherlands, but further research is necessary to determine the effect of specific mastitis pathogens on BMSCC and BMSPC.

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