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Somatic cell count and presence of microbial pathogens in milk of goats in Slovakia

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Research Article

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Abstract

The objectives of the study were to determine somatic cell count (SCC) and evaluate the presence of pathogens (IMI – intramammary infection) in late lactation (LL), followed by the start (colostrum, CL) and approximate peak (established lactation, EL) of the next lactation, as well as to assess the possible transmission of IMI from lactation to lactation. The study was performed on a dairy farm in northern Slovakia. A total of 489 half udder milk samples (242, 80 and 167 in LL, CL and EL, respectively) were collected. Pathogens were identified using MALDI-TOF MS and PathoProof (the latter only in LL). SCC was determined only in LL and EL. Samples were divided according to SCC in four groups from lowest (SCC1 $< 500 \times 10^3$ cells mL^{-1}) to highest (SCC4 $\geq 2000 \times 10^3$ cells mL^{-1}). SCC was higher in LL than in EL. The prevalence of pathogens identified using MALDI-TOF MS was 16.5, 38.8 and 12.6% in LL, CL and EL, respectively. Non-*aureus* staphylococci and mammaliicocci (NASM) were the most common isolated pathogens in goat milk and colostrum. *Staphylococcus (S.) caprae* and *S. epidermidis* species tended to cause persistent IMI in the next lactation. The identification of pathogens using PathoProof was higher than with MALDI-TOF MS. Of all the pathogens (n = 262) identified using PathoProof, the most common were *Staphylococcus* spp. (86.7%) of which 65.8% exhibited the β-lactamase gene. Additionally, *Escherichia coli* (4.2%), *S. aureus* (2.7%), *Enterococcus* spp. (2.3%), *Streptococcus uberis* (1.9%), *Mycoplasma* spp., *Protetheca* spp. (0.8% each), *Arconabacterium pyogenes*/*Peptoniphilus indolicus* and yeast (0.4% each) were also detected using PathoProof. Better identification of pathogen presence in samples with high SCC could contribute to the discussion about SCC as an indicator of subclinical mastitis in goats.

Somatic cell count (SCC) in milk as an immunological response of the udder to the presence of microbial pathogens (IMI – intramammary infection) could be a reliable indicator for detecting mastitis in goats but still remains under discussion and is rarely used as compared to cows. This may be explained by the fact that SCC is influenced also by some physiological factors. Whilst IMI does significantly increase SCC in the milk of goats, many samples with high SCC are free of bacteria (Tvarožková *et al.*, 2023; Smistad *et al.*, 2021). IMI in dairy goats is predominantly caused by non-*aureus* staphylococci and the closely related mammaliicoccal species (NASM) (Rosa *et al.*, 2022). Whilst less prevalent, *Staphylococcus* (*S.*) *aureus* and *Escherichia (E.) coli* also cause clinical caprine mastitis (Rovai *et al.*, 2014; Persson and Olofsson, 2011). In goats, some NASM species are more pathogenic and have higher odds of persistence than others (Bierner Gosselin *et al.*, 2019; Koop *et al.*, 2012). To effectively eliminate and prevent IMI in goats using SCC, specific standards and guidelines for goats are necessary (Smistad *et al.*, 2021; Persson and Olofsson, 2011).

The hypothesis of this work was that the high level of somatic cells in the milk of goats is caused by the presence of mastitis-causing pathogens, which may persist in goat udders during lactation periods. The objectives of the study were to determine somatic cell count in goat milk and evaluate the presence of pathogens in milk in late lactation, followed by the colostrum and beginning of the next lactation, as well as to assess the possible transmission of IMI from lactation to lactation.

Material & methods

Animals

White shorthaired goats from an ecological dairy goat farm (northern Slovakia) were included in the study. The goats were machine milked twice a day in the parlour without udder preparation or postmilking teat dipping. Average milk production was approximately 420 kg/goat/lactation (210 days).

Sampling

Milk sampling at half udder level started in late lactation (LL, n = 242) before the dry period in October. After kidding (March to April), colostrum sampling (CL, n = 80) was performed. The kids stayed with their mothers for 5 to 6 weeks and were allowed to suckle. Mothers were not milked in this period. After 7 to 8 weeks of lactation (1 to 2 weeks after weaning), the last series of milk samples (established lactation, EL, n = 167) were collected. All half udder samples (n = 489) were collected aseptically (3-5 ml in sterile tubes) for bacteriological examination prior to the sampling for SCC determination (30-40 ml in tubes with bronopol preparation) using Somacount 150 (Bentley Czech, USA). The owner of the farm collected some samples only for bacteria identification; therefore, 175 samples from LL and 161 samples from EL were available for SCC determination. Different numbers of samples in the studied periods were due to farm management approaches. For possible transmission of pathogens from lactation to lactation, 31 of the same goats (first group – FG) were sampled in all three periods and 40 goats (second group – SG) in LL and EL only.

Diagnostic procedures

Bacteriological cultivation, identification of bacterial colonies and evaluation of samples were described in the previous study (Tvarožková *et al.*, 2023). The preparation of bacteriological colonies for identification was performed at species level using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) as described by Bierner Gosselin *et al.* (2018). For FG and SG, the transmission of pathogens from lactation to lactation was considered as persistent IMI. In addition to MALDI-TOF MS, the PathoProof Complete-16 kit was used as a highly sensitive method to analyse the presence of 15 mastitis-causing microorganisms and the β-lactamase penicillin resistance gene in staphylococci (including *S. aureus* and all major coagulase-negative staphylococci). Due to the test capacity, PathoProof was used only in LL group of samples where most of them had high SCC. During analysis 4 samples were discharged and thus 238 samples were available. PathoProof Complete-16 kit was used according to the manufacturer's instructions at the VETWELL s.r.o. veterinary laboratory. Briefly, bacterial DNA was extracted from 350 µl of milk samples and eluted with 100 µl buffer AE. Real-Time PCR was performed on Applied Biosystems 7500 Real-Time PCR System. Amplification results were analysed using the 7500 software v2.0.6, which calculates baseline and threshold values based on the assumption that the data exhibit a typical amplification curve.

Statistical analyses

Distribution of samples was evaluated in four SCC groups: SCC1 $<$ 500, SCC2 $\geq$ 500 $<$ 1000, SCC3 $\geq$ 1000 $<$ 2000, SCC4 $\geq$ 2000, all $\times$ 10^3 cells/mL. Analysis of variance was used to evaluate dependence of SCC on stage lactation, where SCC was transformed to somatic cell score (SCS = LOG_2 (SCC/100,000) + 3) to normalize data.

Results

LL exhibited significantly higher SCC than EL in the next lactation (2784 vs 529 $\times$ 10^3 cells/ml, SCS values of 7.80 ± 0.15 vs 5.41 ± 0.16, respectively). Within the four SCC groups (lowest to highest), 8.6, 12.0, 21.7 and 57.7% of the samples were from LL, while 57.8, 16.1, 13.7 and 12.4% were from EL, respectively. In samples that had SCC measured, pathogen presence was observed in 18.9% (33/175) and 13.0% (21/161) of the samples in LL and EL, respectively. From the samples with pathogen presence in LL and EL, there were 87.9 and 66.7% (respectively) in the two highest groups, SCC3 and SCC4.

Table 1. Results of bacterial cultivation of half udder milk samples of goats collected in late lactation and in the next lactation colostrum and established lactation using MALDI-TOF MS

Item	Late lactation	Colostrum next lactation	Established next lactation
All samples	242	80	167
Negative samples (%)	202 (83.5%)	49 (61.3%)	146 (87.4%)
Positive samples (%)	40 (16.5%)	31 (38.8%)	21 (12.6%)
Total of pathogens	40	34	22
S. caprae[3]	15	1	7
S. epidermidis[3]	12	5	9
S. equorum[3]	1	4	
S. warneri[3]	1	1	1
S. simulans[3]	1		
S. capitis[3]		1	
S. cohnii[3]		1	
S. lentus[1,3]		7	
S. vitulinus[1,3]		6	1
Aerococcus viridans[3]		1	
Corynebacterium stationis[3]		1	
Bacillus spp.[2,3]		4	3
Mannheimia haemolytica[3]			1
Not reliable identification[3]	10	2	

[1] *M. lentus* and *M. vitulinus* were referred as *S. lentus* and *S. vitulinus* in the Bruker Biotyper® library
[2] *including Bacillus pumilus* (LL, n = 2), *Bacillus altitudinis* and *Bacillus mycoides* (BL, n = 1 each)
[3] A score values ≥2,0 a given species were considered adequate to be reported at species level. A score between 2.0-1.7 a given species were considered adequate to be reported at genus level. A values below 1.7 were considered for non-identified bacterial species and to be reported as not reliable identification.

Using MALDI-TOF MS, the presence of pathogens was found in 40 (16.5%), 31 (38.8%) and 21 (12.6%) of all samples in LL, CL, and EL (Table 1). The most frequently isolated bacteria were NASM, especially *S. caprae* with *S. epidermidis* in LL (37.5 and 30.0%) and EL (31.8 and 40.9%), and *Mammaliicoccus (M.) lentus* with *M. vitulinus* (20.6 and 17.6%) in CL. In the FG, two goats (6.5%) had persistent IMI with *S. caprae* and *S. epidermidis* in the left teat. Infected goats (n = 8) in LL transmitted IMI to the next lactation (BL) in SG.

Compared to MALDI-TOF MS identification, many more samples were detected as positive (n = 230/238) using PathoProof, where 200 samples contained one pathogen, 27 samples two, and 3 samples three different pathogens. Of all the pathogens (n = 262) identified using PathoProof, the most common were

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Staphylococcus spp. (86.7%), of which 65.8% were *Staphylococcus* spp. with the *β*-lactamase gene. Additionally, *E. coli* (4.2%), *S. aureus* (2.7%), *Enterococcus* spp. (2.3%), *Streptococcus uberis* (1.9%), *Mycoplasma* spp., *Protetheca* spp. (0.8% each), *Arconabacterium pyogenes*/*Peptoniphilus indolicus* and yeast (0.4% each) were also detected. Only 8 samples were identified as negative for the presence of any pathogens. Non-identified pathogens using MALDI-TOF MS (25% of positive samples) were identified using PathoProof as *Staphylococcus* spp. with *β*-lactamase gene (7 samples), *Staphylococcus* spp. (2 samples) and *Staphylococcus* spp. with *β*-lactamase gene, including *Enterococcus* spp. and *E. coli* (1 sample). On the other hand, one of the samples was identified using MALDI-TOF MS but wasn´t identified using PathoProof. Two of the positive samples identified using MALDI-TOF MS (*S. caprae)* did not get the same results using PathoProof.

Discussion

The stage of lactation significantly affected SCC, which is in line with other studies (Tvarožková *et al.*, 2023; Smistad *et al.*, 2021). Therefore, late lactation is considered a risky period for mastitis caused by IMI (Smistad *et al.*, 2021; Koop *et al.*, 2012). Our results show that the frequency of samples with bacterial presence increased with increasing SCC, supporting a possible relationship between SCC and pathogens (Smistad *et al.*, 2021). The limit of SCC as a diagnostic tool for subclinical mastitis is still a subject of discussion among scientists, but most of the research suggests either 500 or 1000 × 10^3 cells mL^{-1} can be associated with IMI (Tvarožková *et al.*, 2023; Smistad *et al.*, 2021). Our results support this, although we also found some samples with high SCC but no pathogen presence, similarly to the findings of Tvarožková *et al.* (2023) and Persson and Olofsson (2011).

The identification of mastitis pathogens contributes to a suitable preventive mastitis program. In our study, the most frequently isolated bacterial species using MALDI TOF MS were NASM, which corresponds to our earlier and other studies (Tvarožková *et al.*, 2023; Smistad *et al.*, 2021; Bierner Gosselin *et al.*, 2018; Koop *et al.*, 2012). To our best knowledge, this is the first study on the detection of pathogens in goat colostrum in Slovakia, where NASM were predominant pathogens. During lactation, *S. caprae* and *S. epidermidis* were predominant species in LL and EL, as in studies by Tvarožková *et al.* (2023) and Koop *et al.* (2012). We also confirmed those species to cause persistent IMI as shown in other studies (Bierner Gosselin *et al.*, 2019; Koop *et al.*, 2012).

The recent introduction of MALDI-TOF MS to routine diagnostics increases knowledge about mastitis-causing bacteria in goats at the species level (Smistad *et al.*, 2021). However, MALDI-TOF MS is culture-based, in which samples with high SCC are frequently bacteriologically negative. One of the explanations could come from the study of Rovai *et al.* (2014). They reported that the secretion of bacteria is not uniform through repeated sampling, and therefore low bacteria count can lead to a negative result, particularly in bacterial culturing, but not using PathoProof. In our study, PathoProof in LL confirmed the presence of pathogens in samples with high SCC. Detection of pathogens in culture-based, bacteriologically negative samples with high SCC could be explained by DNA identification of dead bacteria (Adkins *et al.*, 2017), which should be taken into account when interpreting the presence of bacteria (Rovai *et al.*, 2014). An important findings using PathoProof were firstly, the detection of *Prototheca* spp. which is also classified as the causative agent of contagious mastitis in cows (Adkins *et al.*, 2017), secondly, detection of some pathogens, such as *Mycoplasma* spp. which are difficult to detect using the routine diagnostic methods and thirdly, the most commonly detected bacteria were *Staphylococcus* spp. containing a gene encoding *β*-lactamase, contributing to the evidence of antimicrobial resistance of mastitis pathogens (Rovai *et al.*, 2014). Additionally, a potential explanation for higher resistance to penicillin (β-lactamase gene) could be the fact that antibiotics were used on the farm before ecological production started.

In conclusion, we found many milk samples with an SCC exceeding one million cells/ml, where subsequent cultivation revealed a high frequency of bacterial growth. Using MALDI-TOF MS, the most frequently identified pathogens were NASM, with *S. caprae* and *S. epidermidis* predominantly found in both late lactation and in the next establsihed lactation, and *M. lentus* and *M. vitulinus* in colostrum at the start of the next lactation. *Staphylococcus* spp. containing a gene encoding *β*-lactamase were identified in samples in late lactation using PathoProof. Improved identification of pathogen presence in samples with high SCC could contribute to the ongoing discussion about SCC as an indicator of subclinical mastitis in goats.

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