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Somatic cells and bacteriological examination of milk samples of goats

*Barbora Gancárová[1], Kristína Tvarožková[1], Michal Uhrinčať[2], Lucia Mačuhová[2], Juliana Mačuhová[3], Vladimír Tančin[1,2]**

[1]*Institute of Animal Breeding, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Slovak Republic*
[2]*Research Institute for AnimalProduction Nitra, National Agricultural and Food Centre, Lužianky, Slovak Republic,*
[3]*Institute for Agricultural Engineering and Animal Husbandry, Freising, Germany*
**Corresponding author: vladimir.tancin@uniag.sk*

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Abstract: Mastitis, mainly caused by contagious bacteria, is an important disease in dairy goat production, especially subclinical mastitis. The aim of the research was to study the relationship between somatic cell count (SCC) and presence of mastitis pathogens in goat milk during the period 2022–2024. A total of 987 udder-half milk samples were obtained from 5 goat farms in Slovakia. The presence of pathogens was determined by bacterial cultivation and grown colonies were identified by MALDI-TOF MS at the species level. Fossomatic 7 was used to determine SCC. To evaluate the frequency distribution of the samples, the following groups of SCC were considered: SCC1 < 500 × 10^3 cells/ml, SCC2 ≥ 500 < 1 000 × 10^3 cells/ml, SCC3 ≥ 1 000 < 2 000 × 10^3 cells/ml, SCC4 ≥ 2 000 × 10^3 cells/ml. The individual milk sample were 42.2% in SCC1, 17.0% in SCC2, 16.7% and 24.0% in SCC3 and SCC4, respectively. Of the total number of samples, 23.0% were bacteriologically positive. The most frequently identified pathogens were *Staphylococcus* spp., especially non-*aureus staphylococci* and mammaliicocci (NASM). Among the NASM, *Staphylococcus epidermidis* (40.3%), *Staphylococcus caprae* (27.5%) and *Staphylococcus simulans* (10.4%) were the most prevalent species. Only 2 samples were positive for *Staphylococcus aureus*. A higher occurrence of pathogens was identified in SCC ≥ 1 000 × 10^3 cells/ml (SCC3 and SCC4) compared to SCC < 1 000 × 10^3 cells/ml (SCC1 and SCC2). However, approximately 34.8% of uninfected half udders with no bacteriological findings had SCC ≥ 1 000 × 10^3 cells/ml. In conclusion, NASM were the most common bacterial causative agents of subclinical mastitis in goats and it is not easy to set a specific SCC for subclinical mastitis diagnosis as it is in dairy cows.

Keywords: goat's milk; health; mastitis; pathogens; udder

Mastitis is one of the most serious health and economic problems in dairy goat production, negatively impacting the production, health status, and welfare (Contreras et al. 2007; Podhorecka et al. 2021). Mastitis is divided into clinical or subclinical form based on the visual manifestations

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of the disease (National Mastitis Council 2017). Subclinical mastitis is predominant in dairy goats (Contreras et al. 2007) and is usually associated with increased somatic cell count (SCC) due to the presence of a bacterial pathogen (Bagnicka et al. 2011; Smistad et al. 2021; Tvarozkova et al. 2023). In dairy goats, like in other dairy animals, the detection of subclinical mastitis is mainly based on California mastitis test (CMT), SCC, and bacteriological culture (McDougall et al. 2010; Persson et al. 2014; Kuchtik et al. 2021; Smistad et al. 2021).

Many milk samples show high SCC despite being free of bacteria; however, as SCC increases, the percentage of bacteriologically positive samples also rises (McDougall et al. 2010; Kovacova et al. 2021; Tvarozkova et al. 2023). Further research is needed to explain high SCC in goat milk and to improve methods for detecting pathogens in such milk. In earlier works, Mattila (1985) and Pyorala and Mattila (1987) suggested that the so-called sterile mastitis, characterised by an increase in SCC without bacteriological findings, may be caused by the following factors: (*i*) Infection is present, but inhibitory substances in the milk (phagocytes, lactoferrin, lysozyme, immunoglobulins, etc.) prevent the growth of colonies when the causative organism is isolated. (*ii*) The microorganism is not present in sufficient numbers because it is quickly eliminated by the host. (*iii*) The use of inappropriate laboratory methods may fail to identify agents that cannot be detected by standard bacteriological procedures, such as *Mycoplasma* spp. (*iv*) Injury to the udder and teats. (*v*) Endotoxins released from dead bacteria are inflammatory. (*vi*) The healing process is ongoing, and the microorganism has been eliminated, but the inflammatory reaction still persists.

The aim of the research was to study the relationship between SCC and the presence of mastitis pathogens in goat milk from dairy practice during the three-year period.

MATERIAL AND METHODS

Goat farms and sampling

During the three-year observation period (2022–2024), goat farms (n = 5) were visited regularly or randomly during lactation seasons to collect udder-half milk samples (n = 987). Regular milk sampling was carried out several times during one lactation season or even more, depending on the farm possibilities (on farm 1 n = 328 samples from 118 goats, on farm 4 n = 94 samples from 44 goats, and on farm 5 n = 360 samples from 46 goats). Random milk sampling was carried out only once during the observation period (farm 2 n = 151 samples from 76 goats and farm 3 n = 54 samples from 27 goats). Farms 1, 2, 3, and 5 were located in the north of Slovakia and farm 3 in the east of Slovakia. On the farms, the White Shorthaired breed was dominant (in farm 1, farm 2, and farm 4), or there were crossbreds of White Shorthaired, Brown Shorthaired, and Anglo-Nubian goats (farm 3 and farm 5). On all farms, the goats were machine-milked and only on farm 5 a hygienic milking protocol was used that includes pre-milking disinfection and drying of the teats with clean, single-use hygienic wipes, discarding the first strips of milk, and post-milking teat dipping. Only clinically healthy goats, exhibiting no visible abnormalities in the udder or milk, were included. Milk samples were analysed for the presence of bacterial pathogens and SCC. Samples for bacteriology were collected aseptically in sterile tubes (5 ml of milk), with the initial portions of milk from each teat discarded and the teat ends disinfected with 70% alcohol prior to milk collection. Afterwards, approximately 40 ml of milk were collected in other tubes for SCC determination. According to National Mastitis Council (2017) samples for bacteriology were frozen at –20 °C immediately after collection, using a WAECO portable freezer (Mobicool Electronic, Zhuhai, China), transported to the laboratory, and stored in a freezer until bacteriological procedures were performed within two to three weeks of collection. Samples for SCC determination were preserved with acidiol, stored in a 4 °C cold box, and transported to the accredited testing laboratory for SCC determination within 6 h of collection.

Bacteriological procedures

For bacteriological procedures, 0.01 ml milk samples were aerobically cultivated on Columbia blood agar with 5% ovine blood (MkB test a.s., Rosina, Slovakia) at 37 °C for 24 h and re-evaluated after 48 h of incubation. Bacteria growing on agar plates were routinely identified by morphological and biochemical tests. Species

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identification was performed by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) spectrometry (Bruker Daltonics, Bremen, Germany) according to Tvarozkova et al. (2019). Milk samples were considered positive if at least one colony-forming unit (CFU) of contagious pathogens, such as *Staphylococcus aureus* or *Streptococcus agalactiae*, was detected. In the case of other pathogens, a sample was considered positive if the growth of at least five CFU was observed. The sample was classified as contaminated if more than three different types of colonies were detected with no bacterial growth of contagious pathogens.

Somatic cells

SCC was measured using the method according to STN EN ISO 13366 (Milk, enumeration of somatic cells guidance on the operation of fluoro-opto-electronic counters) with Fossomatic 7 analyser (FOSS, Hillerød, Denmark), equipped with FOSS Integrator software, in the accredited testing laboratory EXAMINALA, Výskumný ústav mliekárenský a.s., Zilina (Dairy Research Institute).

Statistical analysis

The milk samples at the udder half level were divided into four groups based on SCC: SCC1 < 500 × 10^3 cells/ml, SCC2 ≥ 500 < 1 000 × 10^3 cells/ml, SCC3 ≥ 1 000 < 2 000 × 10^3 cells/ml, SCC4 ≥ 2 000 × 10^3 cells/ml using MS Excel (v2012). Analysis of variance was used to evaluate the dependence of SCC on the lactation stage, where SCC was transformed to somatic cell score (Equation 1) to normalise the data.

$$SCS = \log_2 \frac{SCC}{100\,000} + 3 \quad (1)$$

where:
SCS – somatic cell score.

To analyse the association between SCS in milk and the presence of pathogens, as well as between SCS and the presence of specific pathogen groups (groups: *S. caprae*, *S. epidermidis, S. simulans* – the most frequent specific pathogens isolated in our study, other pathogens, and no pathogens: (Tables 1 and 2), a linear mixed model with the PROC MIXED, SAS v9.4 (SAS Institute, Cary, NC, USA) procedure was used. SCS was examined as the dependent variable. The fixed effects included the factors udder half and pathogen factor. To account for the repeated measurements within individual goats and the hierarchical structure of the data, we specified goat nested within farm as a random effect. We applied Tukey's method for multiple comparisons, to compare SCS across pathogen factors. The data are presented as = least squares means ± standard deviation (LSM ± SD).

A generalised linear mixed model (GLMM) was applied using the GLIMMIX (SAS v9.4) procedure to assess the effects of SCC group and udder half on the probability of a positive outcome (pathogen occurrence in the sample). The model specified a binomial distribution with a logit link function for the binary response variable of the pathogen detected (event = '1'). SCC group and udder half were included as fixed effects, while the random effect of individual goats nested within farm was included to account for potential correlations between repeated measures within the same goat. LSM were computed for each level of SCC group and udder half, with adjustments for multiple comparisons using the simulated method to obtain confidence limits and *P*-values. The significance level was set to 0.05.

Table 1. Occurrence of bacterial pathogens in goat milk samples and results of the culture identification by MALDI-TOF mass spectrometry

Items	Number of cultures	
	n	%
Non-*aureus* staphylococcoci and mammaliicocci (NASM)	211	87.2
Staphylococcus aureus	2	0.826
Corynebacterium spp.[1]	8	3.31
Bacillus spp.	4	1.65
Streptococcus spp.[2]	3	1.24
Kocuria rhizophila	1	0.413
Mannheimia haemolytica	1	0.413
Truepella pyogenes	1	0.413
Other unidentified species[3]	11	4.55
Total	242	100

[1]including *Corynebacterium bovis* (*n* = 6), *Corynebacterium stationis* (*n* = 1) and *Corynebacterium camporealensis* (*n* = 1)
[2] including *Streptococcus pluranimalium* (*n* = 2), *Streptococcus dysgalactiae* (*n* = 1); [3]score values range 0.00 to 1.69

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Table 2. Non-*aureus* staphylococci and mammaliicocci (NASM) identified in goat milk samples

NASM	Number of isolates	
	n	%
Staphylococcus epidermidis	85	40.3
Staphylococcus caprae	58	27.5
Staphylococcus simulans	22	10.4
Staphylococcus warneri	14	6.64
Staphylococcus chromogenes	7	3.32
Staphylococcus capitis	5	2.37
Staphylococcus equorum	3	1.42
Staphylococcus horminis	2	0.948
Staphylococcus auricularis	2	0.948
Staphylococcus pasteuri	2	0.948
Staphylococcus cohnii	2	0.948
Staphylococcus petrasii	2	0.948
Staphylococcus arlettae	2	0.948
*Staphylococcus lentus**	1	0.474
Staphylococcus lugdunensis	1	0.474
*Staphylococcus vitulinus**	1	0.474
Staphylococcus microti	1	0.474
*Staphylococcus sciori**	1	0.474
Total	211	100

**S. vitulinus, S. lentus* and *S. sciuri* have recently been reclassified as *mammaliicoccus* species but they still appear in the old taxonomy in the Bruker Biotyper® library for matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

RESULTS AND DISCUSSION

When testing the effect of udder half and positivity of samples (samples with bacterial growth and samples without bacterial growth), no significant differences were observed in SCS between samples from the left and right udder half ($P = 0.517\ 6$). The SCS was significantly lower in milk samples without bacterial growth (5.86 ± 0.10) compared to samples with bacterial growth (6.90 ± 0.14; $P = 0.000\ 1$). When testing the effect of udder half (no significant effect observed; $P = 0.395\ 0$) and the presence of pathogens (*S. caprae, S. epidermidis*, *S. simulans* (the most frequent specific pathogens isolated in our study, other pathogens, and no pathogens), no significant differences ($P > 0.05$) in SCS were found for the presence of pathogens. The SCS values were 6.57 ± 0.24, 7.26 ± 0.22, 7.82 ± 0.44 and 6.58±23 for the presence of *S. caprae, S. epidermidis*, *S. simulans* and other pathogens, respectively. Only the SCS between *S. caprae* and *S. epidermidis* showed a trend toward significance ($P = 0.082\ 9$). However, the SCS differed significantly ($P < 0.05$) in milk samples with the most frequent specific pathogens compared to the SCS in milk samples with no pathogens. In samples containing *S. simulans*, the SCS also showed a trend towards being different from the SCS in milk samples with other pathogens ($P = 0.069\ 5$). Currently, many studies confirm the relationship between the increased SCC and the presence of pathogens in goat milk (Moroni et al. 2005; Rupp et al. 2019; Bezerra et al. 2021; Smistad et al. 2021). Also, a possible effect of bacterial species on SCS was shown in other studies (Paape et al. 2007; Bagnicka et al. 2011).

From the individual milk samples, 42.2, 17.0, 16.7 and 24.0% were categorised as SCC1, SCC2, SCC3 and SCC4, respectively. Our data shows that 40% of samples from the udder half had SCC over a million cells/ml corresponding to the data of Akter et al. (2020), who found out 50% of samples with CMT over 2. However, Kuchtik et al. (2021) reported that SCC in raw milk should rarely exceed 1 000 × 10^3 cells/ml in clinically healthy White Shorthaired goats. Also, in the study of Persson and Olofsson (2011) there was a very low percentage of milk samples from the udder half with high CMT score. Such a high SCC in our trial was related to the farms where samples were collected. The frequency of sample distribution in SCC1, SCC2, SCC3 and SCC4 groups was 32.3, 14.0, 17.7 and 36.0% (farm 1) 17.2, 18.5, 31.1 and 33.1% (farm 2), 14.8, 31.5, 24.1 and 29.6% (farm 3), 25.5, 20.2, 23.4 and 30.9% (farm 4), 70.3, 16.1, 6.9 and 6.7% (farm 5), respectively. The percentage of samples in both SCC3 and SCC4 varies among the farms from 13.6% to 64.2%. The breeding conditions are involved in the udder health (Akter et al. 2020) and therefore they should be under intensive study in our dairy practice.

When analysing the effect of udder half and SCC groups on the probability of bacterial presence in milk samples, only a significant effect of SCC groups was observed ($P < 0.000\ 1$). The probability of bacterial presence (i.e. that milk samples will be positive for bacteria) was similar between the left and right udder half (22.01% and 21.47%, respectively). The probability of positive sample occurrence was 8.48, 21.03, 33.92 and 31.95% in SCC1, SCC2, SCC3 and SCC4 groups, respectively, with no significant difference only

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between SCC3 and SCC4, which supports the above-mentioned positive relationship between SCC and bacterial presence. A high number of samples free of bacteria with high SCC indicates that it is not easy to set a specific SCC that predicts the presence or absence of infection, which is in line with a recent study in goats (Tvarozkova et al. 2023) and other studies showing bacteria-free samples with high SCC (Bagnicka et al. 2011; Smistad et al. 2021). Even Marogna et al. (2012) found out that samples from the udder with at least one clinical sign of mastitis were free of bacteria at 68.3%. On the other hand, the bacterial presence in samples with low SCC in our study was also documented by Bagnicka et al. (2011) and Marogna et al. (2012). However, physiological factors such as parity, stage of lactation, and season need to be considered when setting cut-off values to differentiate infected and uninfected goats (Smistad et al. 2021) as well as factors described by Mattila (1985) and Pyorala and Mattila (1987) mentioned in the introduction. Despite many factors increasing SCC in goat milk, SCC monitoring may represent a valuable tool for assessing the prevalence of subclinical mastitis in goat udders (Rupp et al. 2019). We could support such a view by our results. Therefore, the regular evaluation of SCC on goat farms could be a valuable managerial approach to improve the efficiency of milk production.

From the total number (n = 987) of samples only 23.0% were bacteriologically positive and the growth of 2 bacterial species was recorded in 15 samples. No growth of any pathogens was described in 752 samples and 8 samples were considered as contaminated. The identification success rate using the MALDI-TOF MS was 95.5% (n = 232/242; Table 1) with 11 samples failing to be identified even after multiple repetitions. Many studies have described the use of MALDI-TOF MS as a rapid and reliable method for the identification of microorganisms causing mastitis in cows, sheep and goats. MALDI-TOF MS is becoming one of the most effective methods for veterinary diagnostic laboratories to identify the genus and species of bacteria (Tomazi et al. 2014; Tvarozkova et al. 2019, 2023; National Mastitis Council 2017; Smistad et al. 2021). On the other hand, as it is the case with any biological method, there are limitations which must be taken into account.

The most common bacterial isolations (Table 1, n = 242) were bacteria of the genus *Staphylococcus* spp. NASM and *S. aureus*, (in total 88%), followed by unidentified species like *Corynebacterium* spp., *Bacillus* spp., *Streptococcus* spp., and rare, less abundant bacteria (Table 1). The NASM have been reported in many studies as the most common causative agents of subclinical mastitis, especially in sheep and goats (Contreras et al. 2007; Tvarozkova et al. 2019, 2023; Smistad et al. 2021), which is consistent with our study (Table 1).

The positive result of our study (Table 1) was the negligible occurrence of such an infectious pathogen as *S. aureus* is (n = 2/242, 0.826%). *S. aureus* is a major pathogen frequently causing clinical, subclinical, and gangrenous mastitis in goat udders (Bagnicka et al. 2011). Bezerra et al. (2021) reported that the *S. aureus* infection in goats increases SCC associated with a decrease in goat milk production. Both samples with the above-mentioned pathogens were in SCC4. Some authors observed that the percentage of milk samples from goats with subclinical mastitis caused by *S. aureus* ranged from 4% to 20% (Moroni et al. 2005; Contreras et al. 2007; Bagnicka et al. 2011; Marogna et al. 2012). Subclinically infected goats or sheep could be reservoirs of *S. aureus* for other animals in the herd or flock (Rupp et al. 2019). In addition to the impact on animal health, the consumption of such milk also poses a risk to consumers, as *S. aureus* may contain genes for enterotoxin production (Contreras et al. 2007; Friker et al. 2020).

Corynebacterium spp., especially *Corynebacterium bovis*, was identified in 6 milk samples. These pathogens can be highly contagious and can spread from one cow to another (National Mastitis Council 2017).

Other mastitis pathogens reported in Table 1 are not so common pathogens in goat milk. *Bacillus* spp. is often found as a contaminant in milk cultures and may be an indication of the poor preparation of the teat end before the sample is taken. The presence of these bacteria suggests that the reservoir for the infection is the environment, as they are commonly found in soil, water, dust, air, vegetation, feed, wounds, and abscesses (National Mastitis Council 2017). However, *Bacillus* spp. have been reported as being isolated from the udder of goats (Smistad et al. 2021). The prevalence of *Streptococcus* spp. mastitis was low and *Streptococcus pluranimalium* (n = 2) with *Streptococcus dysgalactiae* (n = 1) were identified at the species level. Compared to the study of Tvarozkova et al. (2023), there were no findings of *Enterococcus* spp. on selected

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Slovak goat farms. The suckling period is critical for the transmission of *Mannheimia haemolytica* from lambs to their mothers, and weaning reduces the frequency of mastitis caused by this bacterium (Contreras et al. 2007). *Kocuria rhizophila* and *Trueperella pyogenes* have been described in goat milk for the first time as udder pathogens in goats in Slovakia. *Trueperella pyogenes* is the bacterium responsible for 'summer mastitis' because summer is usually rich-in-flies, and biting flies may be vectors of the disease (National Mastitis Council 2017).

Table 2 reports NASM identified in goat milk samples. The most common NASM species (Table 2) were *S. epidermidis* (n = 85/211, 40.3%), *S. caprae* (n = 58/211, 27.5%) and *S. simulans* (n = 22/211, 10.4%), although their relative prevalence differed across herds and studies (Bernier-Gosselin et al. 2020; Smistad et al. 2021; Tvarozkova et al. 2023). Traditionally, NASM were considered minor pathogens with lower pathogenicity and slightly increasing SCC compared to major pathogens (Taponen and Pyorala 2009). In contrast, today they are classified as opportunistic pathogens (Turchi et al. 2020). Some species, notably *S. caprae*, *S. epidermidis*, *S. simulans* and *S. xylosus*, have been associated with higher SCS than some other species, as reported by Bernier-Gosselin et al. (2020). SCC increased approximately 3-fold more in goats and sheep than in cows during non-*aureus* staphylococci infection (Leitner et al. 2012). In fact, the high prevalence of these NASM is related to their ability to persist in the mammary gland of goats as previously described by Bernier-Gosselin et al. (2019).

CONCLUSION

With an increase in SCC in udder half milk samples the presence of bacteria was more prevalent. NASM were the most common bacterial causative agents of subclinical mastitis in goats. Higher numbers and probability of occurrence of the pathogens were observed in SCC ≥ 1 000 × 10^3 cells/ml (SCC3 and SCC4) compared to SCC < 1 000 × 10^3 cells/ml groups (SCC1 and SCC2). The high percentage of samples with high SCC and free of bacteria was very often observed and therefore methods for the pathogen identification deserve our attention. Nevertheless, regular monitoring of SCC appears to be an effective prevention tool in distinguishing infected and uninfected goats in the herd.

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Conflict of interest

Authors declare no conflict of interest.

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