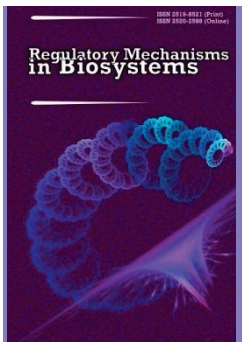

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(2), 292–299
doi: 10.15421/022442

Peculiarities of milk microflora of Saanen goats in the conditions of the Steppe zone of Ukraine

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Article info

Received 12.02.2024
Received in revised form 03.04.2024
Accepted 19.04.2024

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Bilan, M. V., Chumak, V. O., Chumak, S. V., Plys, V. M., & Shkadovska, Y. D. (2024). Peculiarities of milk microflora of Saanen goats in the conditions of the Steppe zone of Ukraine. Regulatory Mechanisms in Biosystems, 15(2), 292–299. doi:10.15421/022442

The quality of goat milk can be affected by mesophilic aerobic and facultatively anaerobic microorganisms (MAFAnM), intestinal bacteria, psychrotrophic, some pathogenic microorganisms, fungi, etc. Traditional microbiological methods, which are used to determine the safety of milk, do not provide an opportunity to obtain an instant result, require the preparation of nutrient media and glassware, which takes up work time and is energy-consuming. We have carried out studies of goat milk by classical (using conventional and selective nutrient media) and alternative modern (using test plates, which are widely used to control microbiological contamination in the food industry) methods. As a result of the conducted research, it was established that the bacterial contamination of goat milk was within the permissible limits. There were no significant differences in the results of using the test plates. According to the classical method, the average indicators of the number of mesophilic aerobic and facultatively anaerobic microorganisms were 4.97 ± 0.14 lg CFU/mL, according to the alternative method – 4.86 ± 0.13 lg CFU/mL. *Escherichia coli* was isolated in four samples (with an average concentration of microorganisms of 1.86 ± 0.62 lg CFU/mL), in the same samples *Enterobacter* spp. in the amount of 1.77 ± 0.61 lg CFU/mL. In three samples, the presence of *Proteus* spp. with average concentration values of 2.19 ± 0.15 lg CFU/mL. Using an alternative method, contamination of milk with *Escherichia coli* (1.53 ± 0.33 lg CFU/mL) was detected in five samples, coliform bacteria were isolated in four samples (1.49 ± 0.50 lg CFU/mL). *Staphylococcus aureus* was not detected by any of the methods, but other representatives of the genus *Staphylococcus* were detected in all samples by both classical and alternative methods (2.48 ± 0.05 and 3.01 ± 0.07 lg CFU/mL). Using the classic method, it was established that two samples were positive for *Enterococcus* spp. (2.46 ± 0.08 lg CFU/mL), *Bacillus* spp. were isolated in six samples (1.70 ± 0.09 lg CFU/mL), in three – *Clostridium* spp. (1.66 ± 0.06 lg CFU/mL), in all six samples psychrotrophic bacteria (2.09 ± 0.26 lg CFU/mL) and yeast (2.41 ± 0.24 lg CFU/mL), four of the samples contained single colonies of mold fungi of the genus *Aspergillus*. Pathogens *Listeria monocytogenes* and *Salmonella* spp. not found. It will be promising to study the dependence of the milk microbiome on environmental factors, as well as the influence of the milk microbiota on the course of technological processes, quality and safety indicators during the production of goat cheese or other dairy products.

Keywords: goat milk; bacterial contamination; safety of milk; determination methods; test plates.

Introduction

In natural human culture, milk and dairy products has long played an important role in nutrition. Currently, it is not only a food product, but also a raw material for the food industry. Apart from cow's milk, goat's milk is gaining popularity due to its benefits: better assimilation of proteins, fatty acids, fat- and water-soluble vitamins and vitamin-like compounds, minerals, lower lactose content and some proteins that can create nutritional problems for children and adults with different reactions. For the production of high-quality uterine and safe products, raw materials with appropriate properties are needed, in particular to the microflora present. That is why bacteriological research on milk from animals found in different areas is topical and relevant (Bhosale et al., 2009; Busol et al., 2015; Sklyarov et al., 2015).

The relatively small number of herds of dairy goats creates the need to accumulate a sufficient volume of goat milk for industrial processing. Therefore, there is a need to monitor changes in the level of microbial contamination of goat milk over a certain period of time. For example, in some regions of the United States, goat milk is collected only once a week. In such cases, the average number of mesophilic bacteria (SPC) can steadily increase to 180,000 CFU/mL after 6 days of storage, exceeding the limit of class A (i.e. 100,000 CFU/mL). The average number of psychrotrophic bacteria steadily increased to 15,000 CFU/mL after 6 days of storage, while the average coliform count was approximately 500 CFU/mL during the first 3 days and 2,500 CFU/mL during 7 days of storage. When stored in refrigerated tanks, milk met Class A criteria for 5 days, but after that the quality decreased due to the growth of psychrotrophic bacteria (Zeng et al., 2007).

In addition, batches of already pasteurized or powdered milk are subject to microbial spoilage. The presence of microbial metabolites can significantly affect the taste properties, shelf life and safety of products. For example, Gram-negative contamination after pasteurization remains a problem for the dairy industry (Murphy et al., 2021). Using PCR, ten types, 119 genera and 249 species of bacteria were identified in dry goat milk samples. *Bacillus*, *Paenibacillus*, *Lactococcus* and *Cronobacter* were the main genera. The dominant species were *Bacillus cereus*, *Lactococcus lactis* and *Cronobacter sakazakii* (Ma et al., 2018). Among cheese samples from Great Britain and France, 82% had satisfactory microbiological quality, 5% were borderline and 12% were unsatisfactory. Four samples (0.6%) were potentially hazardous to health due to the isolation of STEC, >10,000 CFU/g coagulase-positive staphylococci, >100 CFU/g *Listeria monocytogenes* (Willis et al., 2022).

The sanitary and hygienic condition of raw milk is characterized by mechanical impurities, the quantitative and qualitative content of microflora, the presence of pathogens of infectious diseases, etc. There can be several types of milk contamination, both endogenous (the state of the ani-

mal's health) and exogenous (the quality of feed, the cleanliness of the place where the animal is kept, the treatment of the udder before milking, the cleanliness of the hands of workers and milking machines). Due to such a large number of factors, the inspection must be carried out regularly and be of adequate quality, some methods require more time and costs compared to others, so it makes sense to use express methods. Directly, the innovative control methods of the ZM company (USA) meet the requirements of HACCP and allow one to implement and improve the monitoring system. These methods are ATP control of sanitation and hygiene of the enterprise using the Clean-Trace™ system and the microbiological testing system – Petrifilm.

The purpose of the work was: to conduct a microbiological evaluateon of milk from Saanen goats in the conditions of the Steppe zone of Ukraine by classical and alternative (test plates) methods and to determine the peculiarities of its microflora.

Materials and methods

The research was conducted in the laboratory of the Department of Animal Infectious Diseases of the Dnipro State Agrarian and Economic University. 6 samples of milk from goats of the Saanen breed on a farm in Dnipropetrovsk region farm were studied, which were collected immediately after individual milking using sterile utensils according to regulatory documents and transported under the conditions of a refrigerator (4 °C).

Determination of the microflora of raw milk was carried out according to the methods and techniques outlined in the current regulatory and technical documents. Under laboratory conditions, serial ten-fold dilutions of milk were prepared in a sterile sodium chloride solution, and parallel inoculations (1 mL each) of several sample dilutions were carried out in Petri dishes with natural nutrient melted and cooled to + 40–45 °C. Cultivation was carried out under thermostat conditions (+37 °C) for 24–48 hours. According to the results, the number of microorganisms present in the sample was determined as the weighted average value from two counts in successive dilutions. The final results were expressed in colony-forming units (CFU) in 1 mL of the milk sample under investigation. The identification and differentiation of isolated microorganisms was carried out according to the schemes generally accepted in microbiological practice after inoculation on meat-peptone and blood agars, MRS medium, Baird-Parker, Wilson & Blair, Sabouraud dextrose agar (HiMedia Laboratories Pvt. Ltd, India), Enterococcus Agar, Kesler's, Endo's Levin's, Ploskyrev's, Olkenitskyi's, Christensen's medium, Simmons Citrate Agar, Malonate Agar, Hiss's media with various sugars, etc. (LLC Farmaktiv, Ukraine). They were incubated at temperatures of 24, 30, 37, 45 °C for 2–5 days. They were used to study cultural, sucrolytic, proteolytic, reducing properties, the presence of catalase enzyme, and we performed microscopy of smears that were stained according to Gram. The mobility of the isolated microorganisms was determined according to Shukevich (Bilan et al., 2023).

Together with the first method, an alternative method was used to determine the number of MAFAnM, *Escherichia coli*, coliform bacteria and bacteria of the genus *Staphylococcus* according to the instructions for the corresponding test plates. Aliquots (1 mL) were cultured on Petrifilm™ Aerobic Count plates, 3MTM Petrifilm TM Select *E. coli* Count Plate (SEC), Coliform Count, 3M TM Petrifilm TM (STX) system (3M Microbiology, St. Paul, Minnesota, USA) followed by incubation at 30, 35, 37 ± 2 °C for 24–48 ± 2 hours in a horizontal position with the transparent side up. After incubation, all typical colonies were counted and the final results expressed as colony-forming units per mL. Test plate for MAFAnM (Aerobic Count) is a prepared nutrient medium containing modified nutrients for standard techniques, a cold water-soluble gelling agent and a tetrazolium indicator that facilitates colony counting. The *E. coli* Count Plate – is a culture medium system that contains selective agents, nutrients, a cold-water-soluble gelling agent, and an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG). Rapid Coliform Count Plate – rapid tests that include: ready-to-use VRB culture medium (Crystal Violet Agar with Bile and Lactose), a water-soluble gel, a pH indicator for acid detection, and a tetrazolium indicator that facilitates colony counting. The system for rapid counting of staphylococci – allows you to detect bacteria of the genus *Staphylococcus* and identify *Staphylococcus aureus*. It consists of a 3M TM Petrifilm TM (STX) test plate (prepared culture medium) for the rapid enumeration of staphylococci and a disk (allows the establishment of a deoxyribonuclease (DNAse) reaction to confirm *Staphylococcus aureus*. The results were interpreted according to the protocols described in ISO 72183, ISO 4831, 4832, ISO 72182, ANFOR for one 3M Petrifilm test plate of each sample. Accounting of research results and counting of colonies was carried out on plates on which 15 to 300 colonies grew. The obtained result was multiplied by the value of the corresponding dilution and microorganisms were obtained in 1.0 mL of the milk sample. Where the number of colonies was greater than 300, the number was determined by counting the number of colonies in two squares and the arithmetic mean was determined for each plot. After that, it was multiplied by 20 (the area of the circular sowing area). Counting of *Staphylococcus* spp. carried out in the range: no more than 150 red-purple colonies and (or) no more than 300 colonies in total; no more than 150 pink zones.

Statistical processing of the obtained results was carried out in the Statistica program 6.0 (StatSoft Inc., USA).

Results

After studying goat milk by the cup method, we isolated mesophilic aerobic and facultative anaerobes in the range from 34×10^3 to 3×10^5 CFU in 1 mL. The average indicators of the total number of microorganisms in six milk samples were 4.97 ± 0.14 lg CFU/mL. According to the experimental results obtained by an alternative method, with the help of test systems, the total microbial number was determined in the range from 3×10^4 to 3×10^5 CFU/mL (average concentration values 4.86 ± 0.13 lg CFU/mL) (Fig. 1). The calculated numbers of individual groups of microorganisms are shown in the graph (Fig. 2). The correlation coefficient between the results of the two methods is +0.960. Viscous, small colonies, flat and smooth, rough with uneven colony edges, some resembled a drop of water, were noted on dense nutrient media. In the liquid nutrient medium, the formation of wall growth, granular sediment without turbidity was observed. By the classical method of detection of living environments, *E. coli* types were detected in four milk samples out of six (No. 1, 2, 5, 6) with an average concentration of microorganisms of 1.86 ± 0.62 lg CFU/mL. Colonies of bacteria belonging to the coliform group (coliform) were isolated in the same samples, their average number was 1.77 ± 0.61 lg CFU/mL (Fig. 1). Other representatives of the Enterobacteriaceae family were isolated in three samples (No. 1, 3, 6) with average concentration values of 2.19 ± 0.15 lg CFU/mL. After studying the morphology, tincture, cultural and biochemical properties of the isolated microorganisms, representatives of the genera *Enterobacter* and *Proteus* were identified.

On individual test plates, five (No. 1–3, 5, 6) out of six milk samples were contaminated with *E. coli* with mean microbial cell concentration values of 1.53 ± 0.33 lg CFU/mL and four (No. 1–2, 5–6) samples with coliform bacteria – 1.49 ± 0.50 lg CFU/mL (Fig. 3). The calculated bacteria of *E. coli* and coliform bacteria are shown in the graph. The correlation coefficient for the isolation of *E. coli* between the results of the two methods was equal to +0.599, and for the isolation of coliform bacteria – +0.958.

We did not detect *Staphylococcus aureus* on Baird-Parker medium, but *Staphylococcus* spp. were found in all samples, with an average number of 2.48 ± 0.05 lg CFU/mL. In addition, two samples tested positive for *Enterococcus* spp. (2.46 ± 0.08 lg CFU/mL). Similar to the classical method, *Staphylococcus aureus* was not detected by the test systems in any sample, but *Staphylococcus* spp. was isolated in all six milk samples with a concentration of microbial cells of 3.01 ± 0.07 lg CFU/mL ($P < 0.001$, Fig. 1). The correlation coefficient between the results of the two methods is –0.102. The classical method of spore-forming microorganisms of the family Bacillaceae established the presence of representatives of the genera *Bacillus* in all six samples (1.70 ± 0.09 lg CFU/mL) and *Clostridium* in three samples (1.66 ± 0.06 lg CFU/mL). In six samples, psychrotrophic bacteria (average value of 2.09 ± 0.26 lg CFU/mL) and yeast in the amount of $30–2\times10^3$ CFU/mL (2.41 ± 0.24 lg CFU/mL) were found. In addition, the growth of single colonies of mold fungi of the genus *Aspergillus* was established on Sabouraud dextrose agar – in four samples (No. 1–3, 6). It should be noted that in the samples of milk pathogens *Listeria monocytogenes* and *Salmonella* spp. not found.

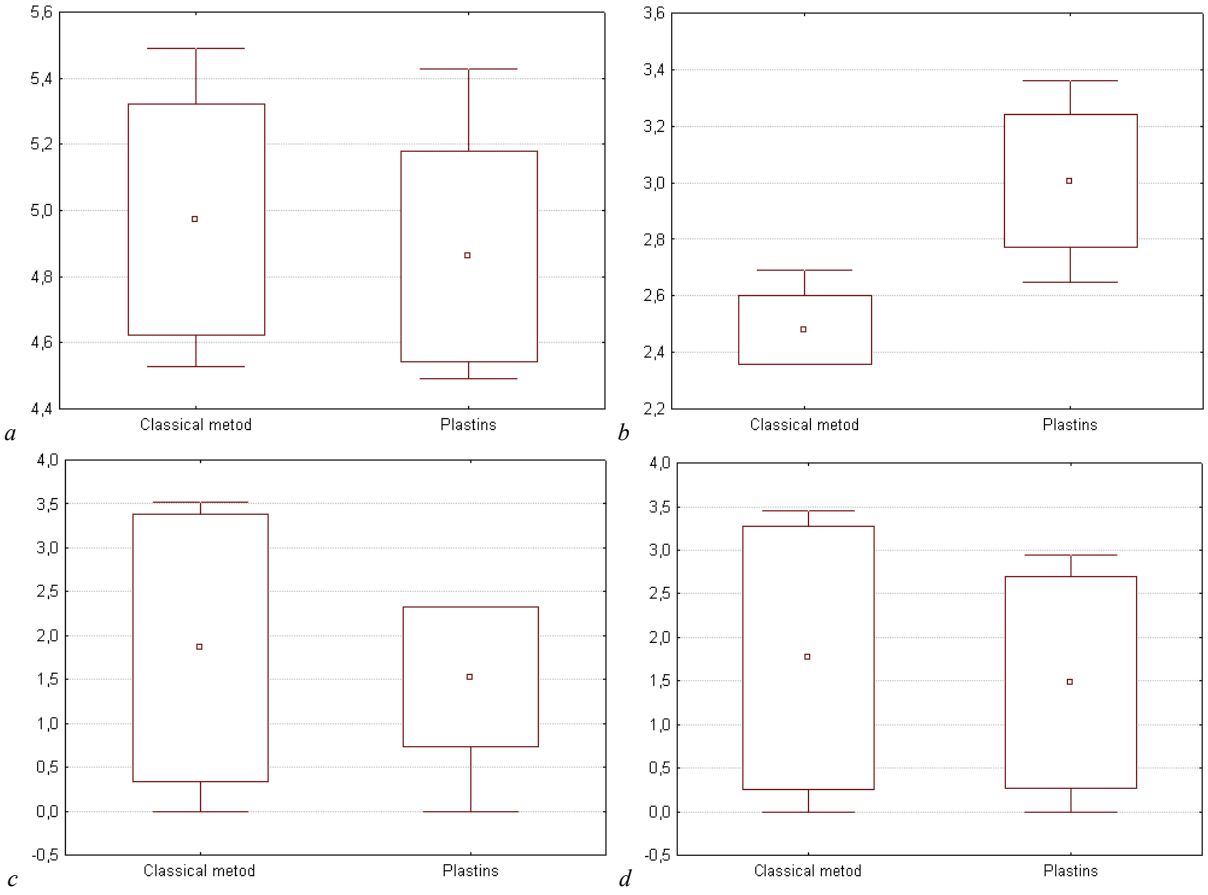

Fig. 1. The number of individual groups of microorganisms (*a* – mesophilic aerobic and facultatively anaerobic microorganisms, *b* – *Staphylococcus* spp., *c* – *Escherichia coli*, *d* – coliform bacteria) isolated from goat milk by the classical method and with the test plates (x ± SD): on the abscissa axis are the names of the research methods; the ordinate axis shows the logarithm ($log_{10}n$) of the number of microorganisms CFU/mL, small squares show the median, the large rectangles show the 25% and 75% quartiles, the vertical lines show 95% of the variation

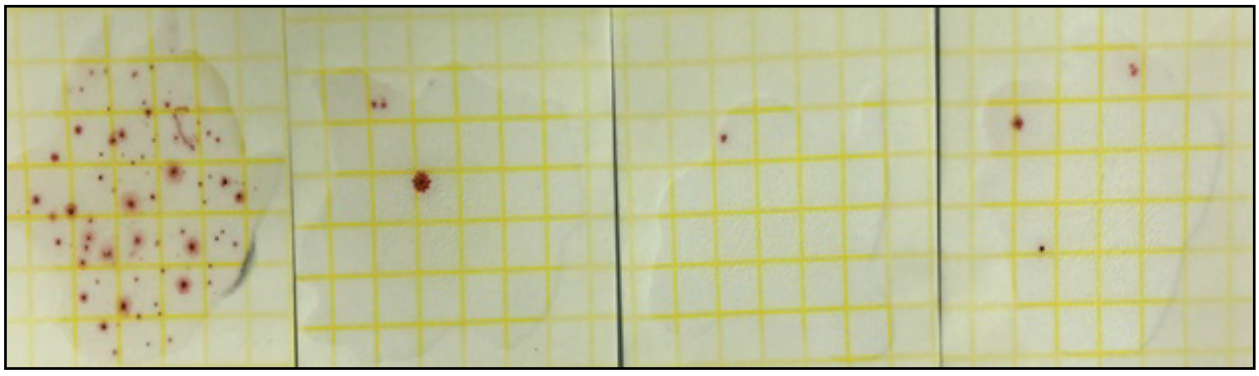

Fig. 2. Determination of bacterial contamination of goat milk using test plates for mesophilic aerobic and facultatively anaerobic microorganisms

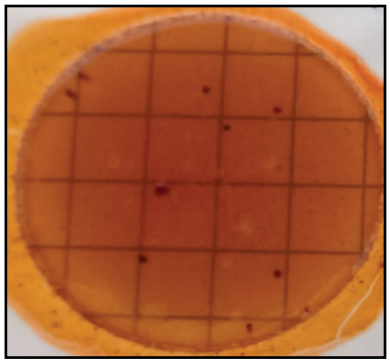

Fig. 3. Growth of coliform bacteria on the test plate Coliform Count Plate – sample No. 1

Discussion

The promising development of goat breeding in our country is indicated by the increase in the number of livestock, 88.5% of which are in auxiliary farms of the population, as of January 1, 2018 (Fedorovych et al., 2022). Replenishment of Ukraine's food supply can be ensured through the development of the goat breeding industry and the use of goat meat and milk in the production of food products (Zazharska et al., 2016; Maslyuk, 2020). The average value of the total number of bacteria in the raw milk of white shorthair goats during the entire lactation fluctuated significantly. Correlations of total bacterial count with protein, fat, lactose, and somatic cell content in milk were insignificant. If hygiene is strictly maintained during the stay, the bacterial contamination is much lower than 500,000 CFU/mL (Kuchtík et al., 2021).

Gecaj et al. (2021) reported microbiological parameters at the end of lactation in raw milk of Alpine and aboriginal red goats in Kosovo.

The total number of mesophilic bacteria in the milk of alpine goats and aboriginal red breed was 250–480 CFU/mL, and coliform and enterobacteria below 100 CFU/mL. A strong positive correlation (0.821) was found for the content of lactose and enterobacteria (EC) in Alpine goats. The coliform group are aerobic and facultatively anaerobic, gram-negative, non-spore-forming bacilli that ferment lactose with the formation of acid and gas at a temperature of 37 °C for 24–48 hours. Basically, these are representatives of the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella* of the Enterobacteriaceae family.

Table 1
Comparison of microbiological counts (mean, CFU or log10 CFU/mL) in goat's milk in this study and other reports

Indicator bacteria	Colony forming units per mL	Features of the sample	References
Mesophilic aerobic and facultative anaerobes	34×10^3 to 3×10^5 CFU/mL	conventional method	This study
	4.97 ± 0.14 lg CFU/mL	conventional method	This study
	3×10^4 to 3×10^5 CFU/mL	alternative method	This study
	4.86 ± 0.13 lg CFU/mL	alternative method	This study
	4.10 lg CFU/mL	raw milk	Rios et al. (2018)
	5.6 lg CFU/mL	bulk tanks	Ramos-Pereira et al. (2019)
	20.000 CFU/mL	pasteurized milk	Ryzhkova (2022)
	4000.000 CFU/mL	raw milk	Ryzhkova (2022)
	3.10–6.40 lg CFU/mL	bulk tank	Álvarez-Suárez et al. (2015)
	4.90 ± 0.70 lg CFU/mL	after milking	Yamazi et al. (2013)
Coliform group	1.77 ± 0.61 lg CFU/mL	conventional method	This study
	1.49 ± 0.50 lg CFU/mL	alternative method	This study
	0.70–5.99 lg CFU/mL	udder-half milk	Taufik et al. (2011)
	0.70–4.45 lg CFU/mL	bulk milk	Andriani & Suwito (2018)
	1.4×10^4 CFU/mL	fresh milk	Gecaj et al. (2021)
	100 CFU/mL	fresh milk	Rios et al. (2018)
	0.3–5.6 lg CFU/mL	raw milk	Ryzhkova (2022)
	310.000 CFU/mL	pasteurized milk	Ryzhkova (2022)
	620000.000 CFU/mL	raw milk	Kyozaire et al. (2005)
	22 CFU/mL	udder-half milk extensive system	Kyozaire et al. (2005)
	15 CFU/mL	intensive system	Kyozaire et al. (2005)
	7 CFU/mL	semi-intensive system	Kyozaire et al. (2005)
	2.70 ± 0.90 lg CFU/mL	after milking	Yamazi et al. (2013)
	2.43 lg CFU/mL	bulk tank	Zeng et al. (2007)
Escherichia coli	1.86±0.62 lg CFU/mL	conventional method	This study
	1.53±0.33 lg CFU/mL	alternative method	This study
	0.65 lg CFU/mL	raw milk	Rios et al. (2018)
	1.40 ± 0.50 lg CFU/mL	after milking	Yamazi et al. (2013)
Enterobacteriaceae	3.10 ± 1.0 lg CFU/mL	after milking	Yamazi et al. (2013)
Enterobacteriaceae family genera *Enterobacter* and *Proteus*	2.19 ± 0.15 lg CFU/mL	conventional method	This study
Staphylococcus spp.	2.48 ± 0.05 lg CFU/mL	conventional method	This study
	3.01 ± 0.07 lg CFU/mL	alternative method	
Coagulase-positive staphylococci	1.70–6.18 lg CFU/mL	udder-half milk	Taufik et al. (2011)
	1.70–5.65 lg CFU/mL	bulk milk	Taufik et al. (2011)
	2.06 lg CFU/mL	raw milk	Rios et al. (2018)
	2.50 ± 0.70 lg CFU/mL	bulk tank	Álvarez-Suárez et al. (2015)
Coagulase-negative staphylococci	1.70–6.41 lg CFU/mL	udder-half milk	Taufik et al. (2011)
	1.70–5.54 lg CFU/mL	bulk milk	
Enterococcus spp.	2.46 ± 0.08 lg CFU/mL	conventional method	This study
Family Bacillaceae genera *Bacillus*	1.70 ± 0.09 lg CFU/mL	conventional method	This study
Clostridium	1.66 ± 0.06 lg CFU/mL	conventional method	This study
Psychrotrophic bacteria	2.09 ± 0.26 lg CFU/mL	conventional method	This study
	4.02 lg CFU/mL	raw milk	Rios et al. (2018)
	3.90 ± 0.70 lg CFU/mL	after milking	Yamazi et al. (2013)
	2.91 lg CFU/mL	bulk tank	Zeng et al. (2007)
Yeast and mold	30–2×10^3 CFU/mL	conventional method	This study
	2.41 ± 0.24 lg CFU/mL		
Proteolytic psychrotrophics	3.60 ± 0.90 lg CFU/mL	after milking	Yamazi et al. (2013)
Total microbial contamination	4.16 lg CFU/mL	bulk tank	Zeng et al. (2007)
	3.74 lg CFU/mL	from udder	Taufik et al. (2011)
	5.69 lg CFU/mL	raw milk	Taufik et al. (2011)
	2.85–3.58 lg CFU/mL	raw milk	Kuchtík et al. (2021)
	250–480 CFU/mL	raw milk	Gecaj et al. (2021)
	1.093 ± 0.401 lg CFU/mL	semi-intensive farm extensive farm	Degirmencioglu et al. (2016)
	4.587 ± 1.604 lg CFU/mL	from udder	Degirmencioglu et al. (2016)
	165.000 CFU/mL	tank of machine milking	Delgado-Pertiñez et al. (2003)
	362.000 CFU/mL	tank of manual milking	Delgado-Pertiñez et al. (2003)
	262.000 CFU/mL	milking machine manual milking	Delgado-Pertiñez et al. (2003)
	16.450 CFU/mL	hand-milking	Kyozaire et al. (2005)
	48.000 CFU/mL	from udder	Kyozaire et al. (2005)
	4.39 ± 0.04 lg CFU/mL	bulk or tank	Delgado-Pertiñez et al. (2003)
	4.89 ± 0.06 lg CFU/mL	machine-milking	Delgado-Pertiñez et al. (2003)
	4.68 ± 0.04 lg CFU/mL	from udder	Delgado-Pertiñez et al. (2003)
	5.12 ± 0.05 lg CFU/mL	bulk or tank	Delgado-Pertiñez et al. (2003)
Lactococcus spp.	5.60 lg CFU/mL	bulk tanks	Ramos-Pereira et al. (2019)
Leuconostoc spp.	4.60 lg CFU/mL	bulk tanks	Ramos-Pereira et al. (2019)
Lactobacillus spp.	4.40 lg CFU/mL	bulk tanks	Ramos-Pereira et al. (2019)

According to taxonomy results, Proteobacteria, Firmicutes, Actinomycetota, and Bacteroidota were the predominant bacteria phyla in both colostrum and mature milk of Saanen dairy goats. In addition, lactation stage noticeably influenced the composition of milk microbiota. Specifically, *Novosphingobium*, *Brachybacterium*, *Psychrobacter*, *Lactobacillus*, *Yersinia*, *Roseateles*, *Cloacibacterium*, *Variovorax*, *Sphingobacterium*, and *Coxiella* were enriched in the colostrum, while *Georgenia*, *Peptostreptococcus*, *Yaniella*, *Planomicrobium*, *Cloacibacterium*, *Azospirillum*, *Turicibacter*, *Cupriavidus*, *Herbaspirillum* and Rhodobacteraceae were the dominant genera in the mature milk (Niyazbekova et al., 2020).

Fourteen isolates were catalase-positive and oxidase-negative, and demonstrated fermentation of substrates specific for *Staphylococcus* spp. Additionally, these isolates were gamma haemolytic and coagulase negative, therefore they were assigned to the group of coagulase-negative staphylococci (CoNS) – 22.6%. One of the isolates was catalase-negative, hence belonging to genus *Streptococcus*. The CAMP test with esculin gave the specific black coloration, which along with lack of growth on MacConkey agar allowed identification of *Streptococcus uberis* (Pamukova et al., 2020).

In addition to the detection of sanitary indicator microorganisms, the presence of dangerous microorganisms that can cause a threat to the health of consumers is monitored (Andriani & Suwito, 2018).

Microbiological contamination of raw milk begins at the farm: microorganisms penetrate from the mammary gland, animal skin and litter, milking equipment, equipment and milk utensils, air, feed, water, hands and clothes of dairy farm workers, other environmental sources (Murska, 2013; Weber et al., 2014; Tonamo et al., 2020). For an objective assessment of the animals' welfare state in terms of climatic conditions, it is advisable to use the definitions of the temperature-humidity index, and to confirm the stress response in dairy goats to any factor of keeping and feeding – laboratory determination of stress biomarkers in the composition of blood, milk, and urine (Chumak et al., 2021).

Alexopoulos et al. (2011) determined that the microbiological quality of sheep's milk is largely influenced by the method of milking, breed, husbandry, season, stage of lactation and farm hygiene. These authors determined the effects of herd size and farm management practices on somatic cell counts and bacterial species in 21 farms in the Xanthi and Evros regions of Northeastern Greece.

The microbiological quality of raw goat milk in Indonesia was investigated for the number and proliferation of indicator bacteria (total microbial contamination, coliforms, coagulase-positive staphylococci, coagulase-negative staphylococci) (Taufik et al., 2011). Skeie et al. (2019) established a change in the composition of *Bacillus* and *Streptococcus* populations in relation to *Pseudomonas* and *Lactococcus* populations, the composition of which did not change.

Doyle et al. (2017) found that milk from stall-fed animals was more likely to contain higher proportions of gut microbes than milk from pasture-fed animals. However, such milk will be more likely to contain more bacteria from the environment. The results of the studies proved a significantly greater diversity of the surface microbiota of udders from animals that were on pasture compared to samples from the surface of udders from animals that were indoors. Milk samples from individual animals that were indoors and did not have their teats treated contained higher relative proportions of, for example, *Eremococcus*, *Ruminococcus*, *Prevotella* and lower proportions of *Pseudomonas*, *Acinetobacter*, *Lactococcus* and *Tumebacillus* compared to milk samples collected from pastured animals. When corresponding milk samples from individual animals were compared with treated udders, 25 genera were found to be present in significantly different proportions in indoor milk samples compared to outdoor milk samples. A greater number (16 genera) of these isolated microorganisms were isolated from milk samples from animals that were housed indoors (*Eremococcus*, *Alloiococcus*, *Trichococcus*, *Prevotella* and *Psychrobacter*). Nine genera including *Flavobacterium*, *Sphingomonas* and *Tumebacillus* were higher in milk samples collected from animals on pasture. There was no significant difference in total bacterial counts between milk samples collected from animals with treated udders ($P = 0.598$), both indoors and outdoors.

Milk produced by Maasai nomads and small-scale urban farmers in Tanzania was analyzed for hygienic criteria (total bacteria, total coliforms, *Escherichia coli* and coagulase-positive staphylococci) and foodborne pathogens such as *Salmonella*, enterohemorrhagic *E. coli* O157:H7 and *Listeria monocytogenes*. A total bacterial count showed that only 67% of raw milk samples and 46% of heat-treated samples met Tanzanian national standards. Milk samples from the traditional milking vessels of Maasai pastoralists had the lowest total bacterial count of around 100 CFU/mL. *E. coli* O157:H7 and *Salmonella* were isolated from 10.1% of raw milk samples, but were not detected in heat-treated or fermented products. Coliform bacteria were isolated in 41% of processed milk samples, indicating a high level of re-infection (Schoder et al., 2013).

In Mexico, about 35–40% of the total milk production on family dairy farms is unpasteurized milk and milk products. 42% of the samples exceeded the limit of aerobic mesophilic bacteria, 83% of the raw milk samples were positive for total coliforms, 54% for fecal coliforms and 46% for *E. coli*. None of the raw milk samples tested positive for *Salmonella enterica*, *Listeria monocytogenes*, or staphylococcal enterotoxin. *S. aureus* was isolated from 9 samples, mycobacteria (*Mycobacteroides chelonae* and *Mycobacteroides abscessus*) from 3 samples (Rios-Muñiz et al., 2019).

Studies in Turkey of the microflora of milk from Saanen goats of extensive and semi-intensive goat farms revealed unequal prevalence of individual species. Milk in samples from semi-intensive farm animals contained most often *Bacillus cereus* (47.8%). In milk from an extensive farm, *Staphylococcus haemolyticus* was the most common isolate (25%). Common types of microflora were *Staphylococcus haemolyticus* 25.0% and 4.3%, *Enterococcus faecium* 12.5% and 4.3%, *E. coli* 9.4% and 4.3%, *Bacillus cereus* 6.3% and 47.8 %, *Bacillus licheniformis* 3.1% and 8.7% in samples from extensive and semi-intensive goat farms, respectively. In the conditions of an extensive goat farm, *Staphylococcus chromogenes* 15.6%, *S. aureus* 9.4%, *S. warneri* 6.3%, *S. caprae* 6.3%, *Bacillus pumilus* 3.1% were detected. *Streptococcus bovis* I (Group D), *Pseudomonas putida*, *Enterococcus faecalis*, *Acinetobacter lwoffii* / *haemolyticus*, *Pseudomonas fluorescens* and *Enterococcus hirae* were detected in milk samples from a semi-intensive goat farm at 4.3% each. *Mycoplasma* and *Brucella* spp. were not detected on any farm (Degirmencioglu et al., 2016).

Microbial contamination of milk from goats on semi-extensive farms in Spain during the production period (from December to September) occurred during the time from leaving the udder to reaching the tank of the cold farm (in the case of machine milking) or bulk tank of the cooperative (in the case of manual milking). Farms with a smaller number of animals (<100 animals) that practiced manual milking had better hygienic and sanitary indicators. No correlation was observed between the number of bacteria and the content of somatic cells (Delgado-Pertiñez et al., 2003). Álvarez-Suárez et al. (2015), reported that the majority of collected goat milk samples from northern Spain (83.8%) complied with the limits of mesophilic aerobes established in the European Union for milk from species other than cows. Isolates of coagulase-positive staphylococci (7.6%) carried staphylococcal enterotoxin (SE) genes of classical types (SEA and SEE). *Cronobacter sakazakii* was not detected in any sample, but enteropathogenic *E. coli* and Shiga toxin producers were. The presence of pathogenic *E. coli* isolates suggests that consumption of raw goat milk may pose a risk to public health.

The influence of the method of milking on the quality of goat milk. Kable et al. (2016) found that milking locations and milking methods, including housing (indoor or outdoor) and type of feed and bedding, alter the bacterial populations present on teats, dust and air in the milking parlor, and ultimately make a contribution to the microbiota of raw milk.

A comparison was conducted of the microbiological quality of milk produced under 3 different types of dairy goat housing systems (intensive, semi-intensive and extensive); the lowest levels of contamination were found among goats under the extensive system (13.3%) compared to contamination levels of 43.3% and 36.7% under the intensive and semi-intensive production systems, respectively. *Staphylococcus intermedius*, *S. epidermidis* and *S. simulans* were the most common bacteria (85.7%) in the milk samples, but there was no significant relationship between SCC and the presence of bacterial contamination in goat milk. To determine the safety of milk produced on small-scale goat farms in Pretoria, the production system in place – machine or hand milking – was identified. Bacteria

were found in 31.1% of the analyzed milk samples. The lowest rate of mammary gland infection was found among goats in the hand milking herd at 13.3%, compared to 43.3% with machine milking. *Staphylococcus intermedius*, *S. epidermidis* and *S. simulans* were the cause of infection in 85.7% of cases, in the rest it was *S. aureus* (Kyozaire et al., 2005).

The microbiota of raw milk can strengthen lactic acid bacteria (contribute to the fermentation of dairy products: *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Propionibacterium* and fungal populations; contribute to health promotion: *Bifidobacterium* and *Lactobacillus*), spoilage bacteria (psychrotrophs, spore-forming putrefactive aerobes and non-spore-forming aerobes); heat-resistant: *Pseudomonas*, *Clostridium*, *Bacillus*, *Proteus*, etc.) and pathogenic microorganisms (*Listeria*, *Salmonella*, *Escherichia coli*, *Campylobacter* and fungi that produce mycotoxins). Microbiota variation is a challenge for the dairy industry. Cultivating milk microbiota at the farm level can also help identify farms with undesirable microbiota and help work with farmers to identify sources of contamination and reduce or eliminate undesirable microorganisms (Coorevits et al., 2008; Fotou et al., 2011; Quigley et al., 2013). Vacheyrou et al. (2011) state that the microflora of raw milk plays an important role in the variety of cheese flavors and can protect against the growth of pathogens. However, milk and milk products may contain various microorganisms and may pose serious health risks. The presence of foodborne pathogens in milk is due to direct contact with sources of infection in the dairy farm environment and secretions from the udder of an infected animal (Alonso-Calleja et al., 2002; Oliver et al., 2005; Ayshpur et al., 2021).

Spore-forming bacteria play an important role in the quality and safety of food products due to their resistance to heat treatment. The presence of heterotrophic mesophilic microorganisms in ultra-pasteurized goat milk and milk drinks was determined using conventional counting and selective methods for the detection of microorganisms from the group of *Bacillus cereus* and *Clostridium perfringens*. The number of heterotrophic mesophilic microorganisms exceeding 10^4 CFU/mL was observed in 80% of batches, bacteria of the *B. sereus* group in 16%, that is, the microbiological quality of products evaluated in Brazilian supermarkets was unsatisfactory (Yamazi et al., 2013; Anjos et al., 2020).

In Ukraine, the requirements for the safety and quality of food products, in particular milk and dairy products, are established in the Law of Ukraine "On Basic Principles and Requirements for the Safety and Quality of Food Products", the Law of Ukraine "On Milk and Dairy Products", Order of the Ministry of Agrarian Policy of Ukraine No. 590 "On approval of the Requirements for the development, implementation and application of permanent procedures based on the principles of the Food Safety Management System (HACCP)", Order of the Ministry of Agricultural Policy of Ukraine No. 118 "On approval of the Requirements for the safety and quality of milk and dairy products" (DSTU 7357:2013).

Compliance with the rules of control at all stages of production, storage, transportation, processing and commercialization will provide the population with high-quality milk and dairy products (Freitas et al., 2009). Determination of the number of mesophilic aerobes and somatic cells are reference methods used as indicators of the quality of raw milk and provide valuable information about the sanitary and hygienic conditions of milking, storage and processing, as well as the detection of pathogenic microorganisms (Freitas et al., 2009; Pantoja et al., 2009).

Classical microbiological methods make it possible to identify microorganisms contained in food products, as well as to count their number. But these methods require a large number of laboratory dishes, a set of nutrient media, conditions to ensure sterility during the research and thermostats for cultivating microorganisms. In addition, the determination of microbiological indicators by classical methods takes quite a long time: it takes up to 3 days to determine the NMAFAnM (the number of mesophilic aerobic and facultatively anaerobic microorganisms) and up to 7 days – a study for the presence of spoilage microorganisms (microscopic fungi and yeast). Methods for determining the presence of microorganisms in samples using dyes have been developed. Rapid microbiological and alternative methods increase the speed or efficiency of isolation, cultivation or identification of microorganisms compared to conventional methods. Currently, considerable attention is paid to the improvement of methods for determining pathogens of bacterial etiology based on accelerated methods of bacteriological analysis. Based on the various properties of microorganisms and their metabolic products, extensive research is being conducted to create modern devices for the indication of microorganisms (Ostapiuk et al., 2010; Lakmini & Madhujith, 2012; Khatsevych & Skladaniuk, 2019).

Evaluation of the ISO 21528-2:2004 Petrifilm™ EB and TEMPO EB systems for the enumeration of Enterobacteriaceae in milk was performed in Brazil with the aim of developing alternative microbiological methods for obtaining rapid results in the food production process in samples of pasteurized and ultrapasteurized cow's milk. Studies of the Petrifilm™ EB method and ISO 21528:2 regression analysis showed a high correlation between samples, r = 0.90 for the microflora of pasteurized milk, r = 0.98 for artificially contaminated pasteurized milk and r = 0.99 for artificially contaminated ultrapasteurized milk. No statistically significant differences were observed between the different methods, so the Petrifilm™ EB system and the TEMPO® EB system can be an alternative to ISO 21528-2:2004 for the analysis of milk for Enterobacteriaceae due to ease of operation and reduced time (Cirolini et al., 2014).

Samples of pasteurized milk were analyzed by scientists in Brazil by counting coliforms at +35 °C and at +45 °C, as well as *Escherichia coli*. A high correlation was found between the methods for counting coliform bacteria at +35 °C, but a low correlation was found for counting coliforms at +45 °C and *E. coli*. The Petrifilm TM and TEMPO® systems showed satisfactory results for coliforms at +35 °C in pasteurized milk, but low results for other microorganisms compared to the traditional enumeration method (Cirolini et al., 2013).

Blackburn et al. (1996) demonstrated that Petrifilm enumeration of mesophilic aerobic and coliform bacteria is a practical and accurate alternative to standard enumeration methods in a wide range of food products, with the advantages of saving time, labor and room in the thermostat. The Petrifilm plate method is simpler, requires less time for sample preparation, and is faster in research than the traditional method (Park et al., 2001).

Good correlation indicators were obtained and the absence of significant differences between average values established when studying raw and pasteurized milk samples using the classical method. However, with the use of Petrifilm Aerobic Count plates, good correlation indicators and the absence of significant differences were observed only when examining raw milk samples. The microbiota of pasteurized milk negatively affected the performance of the Petrifilm Aerobic Count plates, probably due to the presence of microorganisms that adversely affect the indicator of this system (Freitas et al., 2009).

Studies by Rios et al. (2018) of goat milk using Petrifilm™ AC in farms of Parana (Brazil) established average concentrations of mesophilic aerobes, coliforms, *E. coli*, coagulase-positive staphylococcus and psychrotrophic microorganisms, which were 4, 10, 2.38, 0.65, 2.06 and 4.02 lg CFU/mL, respectively. *L. monocytogenes* and *Salmonella* spp. were not detected in the samples. Thus, the high number of coliform and psychrotrophic microorganisms in gray goat milk indicated poor hygiene at the time of milking in Parana farms. Fluctuations in the values of physicochemical data characterize the research conducted to determine the parameters that reflect the Brazilian conditions of goat milk production.

Research proved that goat milk contains a smaller amount of foreign (NMAFAnM, coliforms and *E. coli*) microflora, as well as the number of somatic cells that produce its higher food safety compared to cow's milk. The explanation that goat's milk is less contaminated with foreign microflora than cow's milk is caused by a different diet, the way goats are kept, the peculiarities of milking, the biological animal or the high content of bactericidal substances in goat's milk. By comparing the classical (seeding in a Petri dish) and alternative research methods, a higher economic efficiency of the method for determining the microbiological purity of milk and dairy products using Petrifilm™ plates was established. The number of microorganisms determined by the two methods did not differ significantly – the correlation coefficient for MAFAnM was 0.89, and coliforms – 0.88. The difference between the indicators was within the confidence interval at the level of probability $P < 0.05$ (Ryzhkova, 2022).

A study of the microbiological composition of goat milk samples from northwestern Spain revealed that mean values for mesophilic aerobic microorganisms (Standard Plate Counts, SPCs) were higher, very similar to counts on M17 agar (*Lactococcus* spp.) and higher than the counts on MSE agar (*Leuconostoc* spp.) and on MRS agar (*Lactobacillus* spp.). De-

pending on the season, no statistically significant differences were found (Ramos-Pereira et al., 2019).

The number of aerobic microorganisms in pasteurized milk from Brazil using Petrifilm TM AC and Simplate TM TPC with conventional methods was reported to depend on the nature of the indigenous microbiota. Correlation indices for Petrifilm TM AC counts were "good" but "poor" when using Simplate TM TPC (Tavolaro et al., 2005).

The number of mesophilic aerobes (MA) is the main quality and hygiene parameter for raw and pasteurized milk. Raw and pasteurized milk samples were analyzed using Petrifilm Aerobic Count agar plating and subsequent incubation according to 3 official protocols: IDF/ISO (incubation at +30 °C for 72 hours), American Public Health Association (at +32 °C for 48 hours) and the Ministry of Agriculture of Brazil (at +36 °C for 48 hours). Correlation and absence of significant differences between averages were observed only for raw milk samples. The microbiota of pasteurized milk negatively affected the performance of Petrifilm Aerobic Count plates, probably due to the presence of microorganisms that weakly affect the dye of this system (Freitas et al., 2009; Quigley et al., 2011).

A statistical comparison of spiral (SPPLC) and standard count (SPC) methods of mesophiles, lactococci, leuconostococci, lactobacilli, micrococci, enterobacteria, fungi and yeasts in goat milk and cheese during its production and ripening was carried out. Mean values for the SPLPC and SPC methods differed by less than half a log cycle for all microbial groups studied (from 20.1386 for mesophyll to 10.4397 for *Lactobacillus*). In general, the results of the SPLPC method were favorable compared to the results of the SPC procedure for the enumeration of microorganisms in goat cheese throughout its production and ripening process. However, the suitability of the SPLPC method depends mainly on the investigated microbial group (Alonso-Calleja et al., 2002).

Conclusions

For the first time, the milk microbiota of Saanen goats was determined in the conditions of the Steppe of Ukraine using classical and modern Rapid Petrifilm methods. No significant differences were found in the results of the two methods. The cup method established that the number of mesophilic aerobic and facultative anaerobes in milk was determined at the level of 4.97 ± 0.14 lg CFU/mL, and the method using Petrifilm plates – 4.86 ± 0.13 lg CFU/mL.

According to the results of bacterioscopy and bacteriological diagnostics, the isolated cocci and rods were assigned to the genera *Lactococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Lactobacillus*, *Pseudomonas*, *Bacillus*, *Clostridium*, *Escherichia*, *Enterobacter*, *Proteus*. The obtained results were within the permissible level of microorganisms specified in the current regulatory and technical documents (≤100 – ≤500 thousand CFU/mL).

The obtained results make it possible to assess possible threats to consumer safety at the control points of production and processing of dairy products according to the implementation of the HACCP system. Prevention of microbiological contamination of milk during milking will significantly improve the possibility of storing and obtaining safe products, but the threat of secondary contamination remains.

The authors declare no conflict of interest.

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