Evaluation of goat milk microflora in farms from Transylvania

Cristiana Ştefania NOVAC, Sanda ANDREI, Ioana Adriana MATEI, George Cosmin NADĂŞ, Flore CHIRILĂ, Cosmina Maria BOUARI, Nicodim Iosif FIŢ

University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur street., 400372, Cluj-Napoca, Romania

sandrei@usamvcluj.ro

Abstract

Mastitis represents the inflammation of the mammary gland and is considered to be a serious disease due to the fact that it leads to the decrease of milk yield, with severe consequences on milk quality, increase of animal culling and therefore major economic losses. The aim of this study was to identify and describe the main microorganisms, as well as analyzing the Colony Forming Units in raw goat milk by collecting samples from 18 dairy goat farms from Transylvania, as follows: 6 farms from Alba County, 6 from Sălaj County and 6 farms from Cluj County. The milk samples were taken twice from each farm, gathering a total of 36 samples. The results showed that among Gram positive bacteria, 63.8% of the total number of samples were positive for Staphylococcus spp., 36.1%, for Micrococcus spp., 25% for Streptococcus spp. and in 19.4% of the milk samples was identified Bacillus spp. Other Gram positive microorganisms were isolated, but with a lower frequency: Kocuria spp. (8.33%), Aerococcus spp. (8.33%), Corynebacteruium spp. (5.55%) and Enterococcus spp. (2.77%). Results regarding the Gram negative bacteria showed that E. coli was the most frequently isolated microorganism, with a percentage of 38.9, followed by Klebsiella spp. with 25%, Hafnia spp. with 11.1%, Aeromonas spp. with 8.33% and Raoultella spp. with 5.55%. Data concerning the total number of germs showed that raw goat milk from the Transylvanian farms included in the study fulfils the hygiene criteria in the legislation.

Keywords: milk, goat, microflora, bacteria.

Introduction

Mastitis is defined as inflammation of the mammary gland tissue which occurs in response to various external factors such as intramammary infections, mechanical or thermal injuries. From a clinical point of view, mastitis is classified in two categories: subclinical mastitis (without visible clinical signs) and clinical mastitis (in which visible, local and even general symptoms occur, along with significant changes in milk secretion) (1). Regardless of animal species, udder diseases are considered to be limiting factors in the development of the dairy industry, which lead to major economic consequences due to significantly decreased milk production, poor milk quality and expensive veterinary treatments, severely affected animals being, most often, slaughtered (3,5). Moreover, the importance of the hygiene and safety criteria of milk and dairy products are worth to be mentioned, with the risk of illness occurring among consumers, due to the consumption of possibly contaminated products. Nevertheless, the legal aspects regarding the microbiological quality of milk should be implemented and respected accordingly (8).

An important aspect regarding the udder health is the concept of microbiota of mammary gland. In recent years, the sterility of healthy mammary tissue has been questioned and it has been disapproved by the results of several studies based on bacterial DNA. Therefore, research conducted on bovine milk confirmed the presence of an intramammary normal flora in the healthy udder composed of a variety of bacteria. Furthermore, it is stated that inflammation would be, most often, a consequence of dysbiosis of the microbiota rather than a primary infection (2,6,9). In order to confirm this hypothesis, it has been shown that the microbial flora in milk samples from healthy cows is different from that of cows diagnosed with mastitis. Moreover, the presence of several bacterial species such as *Staphylococcus aureus* and *Streptococcus uberis* in healthy bovine milk samples, with a low number of somatic cells, reinforced the idea according to which bacteria that

is normally found on the skin or intestinal mucosa are also a part of the commensal flora of the mammary gland (6). Despite the fact that numerous studies on bovine milk microflora have been published, research on goat milk is limited and sometimes contradictory. Based on these considerations, the purpose of the present study was to describe the overall goat milk microflora, but further reasearch is needed in order to fully understand and identify the bacterial population in the goat mammary gland.

Materials and methods

The study evaluated the microbiological quality of goat milk samples from 3 counties in Transylvania, with 2 successive samplings at 3 weeks interval. Bulk milk samples were evaluated from 6 farms in Sălaj County, 6 from Alba County and another 6 from Cluj County, with 18 milk samples per collection and a total of 36 included in the study. Sterile containers were used and appropriate temperature levels were conferred during the transport to the laboratory of microbiology, Faculty of Veterinary Medicine Cluj-Napoca.

The cultural examination was carried out after diluting 10 µl of milk in 990 µl of saline. One loopful of diluted milk was used to streak a blood agar Petri dish. The rest of the solution (1000 µl) was used to flood a Mueller Hinton Petri dish, removing the excess fluid. The plates were incubated for 24 hours at 37°C, examining the cultural and morphological characters of bacterial colonies and the presence of haemolysis areas.

Isolated colonies were identified by microscopic examination and biochemical characters. Gram staining technique was used for the preliminary identification of isolated colonies and biochemical characters were evaluated using API 20 Biomérieux system (Bio Mérieux, France) and Vitek 2 technique. Mueller Hinton agar plates were evaluated and colonies were counted using the automatic colony counter CC-1 and colony forming units (CFU) were calculated for each milk sample included in the study.

Results and discussions

A total number of 36 samples (100%) were positive for bacterial growth, with a higher prevalence of Gram positive species, most of these identified as cocci and rods, with Gram negative bacteria less frequently isolated. The identification of milk samples and their association with county origin and sampling interval was designated using the acronym CJ for Cluj County and 1 for the farm number for each county.

The results obtained following the analysis of cultural characteristics and the bacteriological examination showed that *Staphylococcus* spp. was the most frequently isolated Gram positive bacteria, followed by *Micrococcus* spp., *Streptococcus* spp. and *Bacillus* spp. Other Gram positive microorganisms were identified in the milk samples, but with a much lower frequency, such as: *Kocuria* spp., *Aerococcus* spp., *Corynebacterium* spp. and *Enterococcus* spp. On the other hand, *E. coli* had the highest prevalence among Gram negative isolates, followed by *Klebsiella* spp., *Hafnia* spp., *Aeromonas* spp. and *Raoultella* spp. with the lowest number of positive samples. In figure 1, the genera identified exceed 100% due to bacterial association, most frequently among *Staphylococcus* spp., *E. coli* and *Micrococcus* spp.

Table 1. The presence of Gram positive and Gram negative bacteria for each sampling, county and farm. Legend: AB – Alba County, CJ – Cluj County, SJ – Sălaj County

|	Genus	Sampling nr. 1	Sampling nr. 2
Gram positive	*Staphylococcus* spp.	AB1,AB2,AB4,AB6,CJ1,CJ2, CJ3,CJ4,CJ5,SJ1,SJ2,SJ3,SJ4, SJ5	AB1,AB2,AB6,CJ4, SJ1,SJ2,SJ3,SJ5,SJ6
	Micrococcus spp.	SJ1,SJ2,SJ3,SJ4	AB3,AB6,CJ1,CJ2,CJ4,CJ5,CJ6,SJ1, SJ2
	Streptococcus spp.	AB3,CJ1,SJ2	AB1,AB2,AB3,AB4,CJ1,CJ3
	Bacillus spp.	AB1, AB5, SJ2	AB5,SJ3,SJ5,SJ6
	Kocuria spp.	SJ5	SJ3,SJ4
	Aerococcus spp.	SJ6	SJ5,SJ6
	Corynebacterium spp.	CJ1	CJ2
	Enterococcus spp.	CJ3	-
Gram negative	*E. coli*	AB3,CJ1,CJ2,CJ4	AB1,AB2,AB4,CJ1,CJ2,CJ3,CJ4,CJ6, SJ1,SJ2
	Klebsiella spp.	AB2,AB3,AB4, CJ3,CJ5,CJ6,SJ1	AB3, SJ3
	Hafnia spp.	CJ5	CJ5,SJ5,SJ6
	Aeromonas spp.	SJ3,SJ4	SJ4
	Raoultella spp.	SJ5,SJ6	-
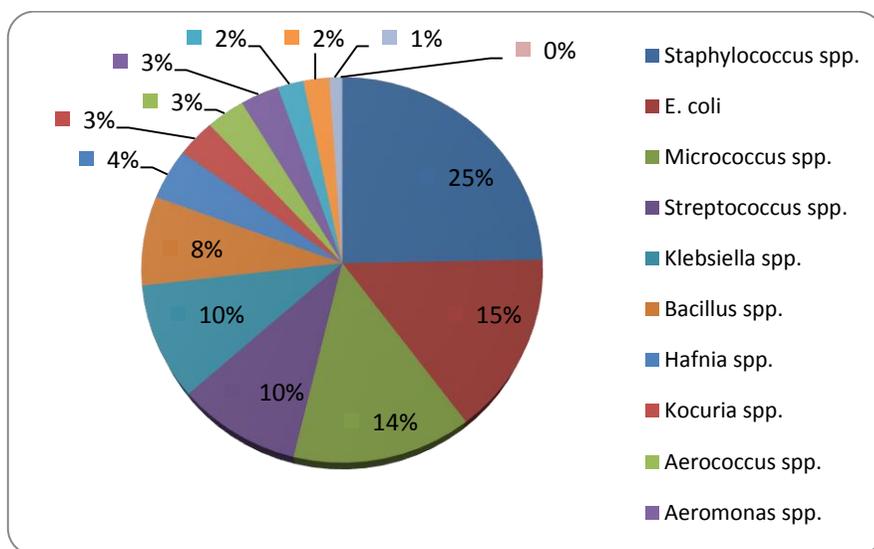
Fig.1. The overall percentage of bacterial genera identified from the total number of milk samples evaluated

Following the initial identification of bacterial colonies and the isolation of pure colonies, the biochemical characters were evaluated using the API 20 Biomérieux system (Bio Mérieux, France), as well as the Vitek 2 technique according to the protocols. Therefore, the following Gram positive species were identified: *Staphylococcus xylosus, Staphylococcus epidermidis*, *Kocuria varians*, *Kocuria rosea* and *Aerococcus viridans,* while the Gram negative tested strains have been identified as *Hafnia alvei, Raoultella planticola* and *Aeromonas sobria.*

275

Table 2. The average of CFU for each farm (1-6), sampling and county

Farm nr. / Average CFU/ml	1	2	3	4	5	6
Alba	27547.75	244087.1	154850.6	211411.6	282024.5	72519
Cluj	150356.5	11900	42350	139486	300713.4	194936.5
Sălaj	7050	34862.8	93038	118513.7	57434.7	289637

The results regarding CFU values in our study for the 36 milk samples had lower values compared to the accepted standard of CFU present in the European legislation for raw milk other than cow milk intended for human consumption (Reg. CE 853/2004). The proportion and genera identified in our study are consistent with the results obtained by Gelasakis et al. (3) and Marogna et al. (5). *Staphylococcus* spp. was the most frequently isolated, followed by *E. coli, Streptococcus* spp. and *Bacillus* spp., similar to the results obtained by Gosselin et al. (4).

Conclusions

The study of identification of microbial flora from raw goat milk in farms from Transylvania concluded that:

➢ Both Gram positive and Gram negative bacteria have been identified, with the predominance of Gram positive species;

➢ The milk samples from Alba County had a higher number of CFU/ml compared to the other two counties included in the study;

➢ Individual CFU values of the present study did not exceed the upper limit of the accepted number of CFU/ml according to the official regulation for raw milk from animal species other than bovine (Reg. CE 853/2004).

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