THE INCIDENCE OF COAGULASE-POSITIVE STAPHYLOCOCCI (*Staphylococcus aureus* AND OTHER SPECIES) IN RAW GOAT MILK COLLECTED DURING DIFFERENT SEASONS

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Abstract. The growth and multiplication of pathogenic bacteria in raw milk depend on various factors including animals` health and the environmental, hygienic and technological conditions during production. In this study, the incidence of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) in raw goat milk collected each season (spring, summer, autumn) from a herd of goats bred in extensive system was monitored. The average growth rate of pathogens in milk was of 1.4×10^3 CFU/ml in the spring months when milk production was high, also with an increase in the contamination degree during summer of 3.2×10^3 CFU/ml, while in autumn the microbial population was quantified at 2.8×10^3 CFU/ml. The average results obtained in the spring months showed a low standard deviation. Seasonal variation indicated a higher contamination during summer in higher temperature conditions, but the 10^5 CFU/ml threshold considered relevant to the possibility of bacterial enterotoxins production was not exceeded. The microscopic examination performed on typical colonies revealed the presence of the specific cluster shape for staphylococcus bacteria. Multiplication of staphylococci in milk occurs by not following appropriate hygiene measures in correlation with environmental factors specific to each season. These germs were analyzed because their presence in the raw material (raw goat milk) influences the hygienic quality of the finished product.

Keywords: hygiene, milk raw, stapylococ, season.

INTRODUCTION

Goat's milk has been feeding people since the earliest times beginning with the first activities of animal's domestication and breeding. The benefits of drinking goat's milk, as well as its pure nutritional value, have increased consumption of dairy products in the recent years. However, the safety of cheeses produced using raw milk is conditioned by many factors, due to the high risk of contamination with pathogens. Among the pathogens with health incidence due to ingestion of contaminated food is *Staphylococcus aureus* a very common organism capable of producing several enterotoxins that cause intoxication symptoms of varying intensity in humans [24]. *Staphylococcus aureus* is one of the most prevalent bacterial pathogens causing food-borne disease worldwide [6].

Staphylococcus bacteria, especially *Staphylococcus aureus*, are one of the main etiological agents involved in milk contamination and foodborne human infections [25]. Staphylococcal enterotoxins can be produced in food by many strains of *S. aureus* and by some other coagulase-positive staphylococci, e.g. *Staphylococcus intermedius*, *Staphylococcus hyicus*, *Staphylococcus delphini*. The enterotoxigenic strain needs to grow to levels $>10^5$ cfu/g before the toxin is produced at detectable levels [7, 21]. Enterotoxigenic *Staphylococcus aureus* strains are able to produce thermostable enterotoxins, that, when ingested, cause nausea, vomiting and diarrhea [16]. Staphylococcal toxin thermoresistance has considerable practical significance because known pasteurization methods are ineffective, while *Staphylococcus aureus* is destroyed by common pasteurization methods [3]. A very important factor in order to maintain food safety is the control of milk temperature because the permissive temperature for the growth and production of toxins by *S Staphylococcus. aureus* is between 6 °C and 46 °C [17].

The maximum content of *Staphylococcus aureus* with $m = 10^4$ CFU·g^{-1} and $M = 10^5$ CFU·g^{-1}, (n = 5, c = 2, where n - the number of units containing the sample, c - sampling which gives values greater than m or between m and M) is a criterion for the hygiene of the process for cheeses made from raw milk in accordance with Regulation (EC) No. 2073/2005 [7].

The purpose of this study is to determine the incidence of raw goat milk contamination with positive coagulase staphylococci and implicit with *Staphylococcus aureus*, depending on the season when the manual milking is practiced, in order to obtain traditional dairy products complying with food safety standards.

MATERIAL AND METHODS

Each season (spring, summer and autumn), 9 samples of milk were collected in order to determine the degree of raw goat milk contamination with positive coagulase staphylococci (*Staphylococcus aureus* and other species). Milk samples were aseptically retrieved in the morning and transported to the laboratory in less than 24 hours under refrigeration conditions. The presence of potentially pathogenic microorganisms has been investigated by the Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), Part 1: Technique using Baird-Parker agar medium according to SR EN ISO 6888-1: 2002 Standard [28]. Five successive decimal dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made. A sample of 1 ml

from each dilution is dispensed in approximately equal amounts on the surface of 3 Baird-Parker agar boxes and dispersed on the surface of the medium.

After incubation at 37 °C for 24-48 hours, only those plates containing up to 300 colonies of which 150 typical and/or atypical colonies at two successive dilutions are taken into consideration. Typical colonies are black or gray, bright and convex surrounded by a clear area. Atypical colonies are brilliant black with or without a narrow white border, the clear area is absent or poorly visible and the opalescent ring is absent or poorly visible, or may be gray colonies without clear areas. To make an estimate of the lowest number of coagulase-positive staphylococci, all plates containing typical and atypical colonies are retained. The count of the N number of coagulase-positive staphylococci present in the sample is as follows:

$$N = \frac{\Sigma a}{V\left(n_1 + 0.1n_2\right)} \cdot d$$

Σa – the sum of the coagulase-positive staphylococcal colonies identified on all the selected plaques;

V – the volume of inoculum applied on each plate;

n_1 – number of plates selected at first dilution;

n_2 – the number of plates selected at the second dilution;

d – the dilution degree corresponding to the first selected dilution.

Thus, the mean values of the number of Coagulase Positive Staphylococci (CPS) colonies (CFU) (mainly *Staphylococcus aureus*) recorded each month of the milking season under variable temperature conditions were calculated. Along with calculating the average of the CPS number, the standard deviation was calculated using the Microsoft Office 2007 (Microsoft Excel) statistical tools. Microscopic analysis of a typical bacterial colony was performed using a Kruss Optronic (Germany) MBL 2100-5 trinocular microscope with the Nikon Coolpix P5000 Digital Camera. The examination was done with the 40x objective.

RESULTS

In goat milk analyzed in spring, summer and autumn, the degree of CPS contamination (*Staphylococcus aureus* and other species) records the values from Table 1. Also, the arithmetic mean of the values recorded in each season, as well as the standard deviation of the values obtained monthly was determined.

The images of the Petri dishes containing the samples after the incubation time are presented in the Fig. 1.

Table 1. Average values and standard deviation of the number of coagulase-positive staphylococci/ml goat milk depending on the season

Season		CFU/ml×10^3			
		S1	S2	S3	$\bar{X} \pm S_{\bar{X}}$
Spring	March	1.38	1.42	1.3	1.4±0.0957
	April	1.32	1.26	1.55	
	May	1.44	1.42	1.51	
Summer	June	3.44	2.4	2.9	3.2±0.3806
	July	3.38	3.05	3.58	
	August	3.6	3.3	3.15	
Autumn	September	2.93	2.85	2.93	2.8±0.1302
	October	2.85	2.8	2.9	
	November	2.75	2.58	2.61	

Note: CFU/ml (colony-forming units per milliliter), $\bar{X} \pm S_{\bar{X}}$ (average ± standard deviation of the average)

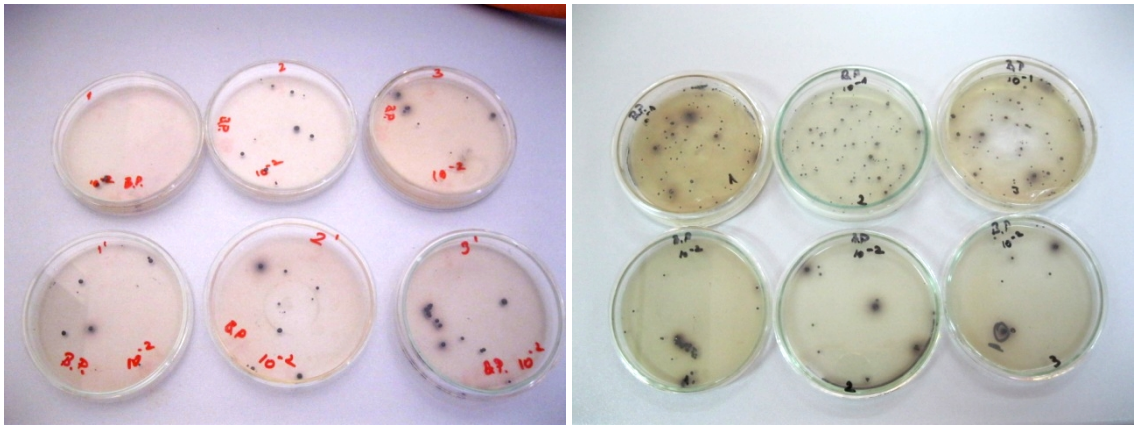

Figure 1. Petri dishes (dilution 10^{-2}) with colonies of coagulase-positive staphylococci

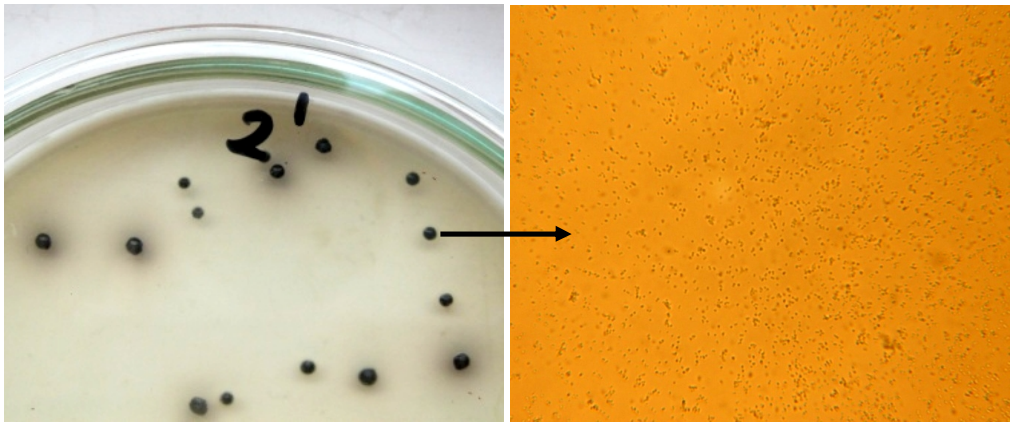

Figure 2. Macroscopic and microscopic aspects of the *Staphylococcus sp*.

As one can see, the macroscopic appearance of the colonies developed on Baird Parker Agar Base with Egg Yolk Tellurite Emulsion is typically black. Typical colonies of *S. aureus* on this medium are of 2-5 mm diameter with an opaque halo as a result of lipase activity and a clearing zone due to the proteolytic action [26].

Microscopic analysis by performing "in vivo" preparations on typical CPS colonies allowed the observation of the specific cluster shape of *Staphylococcus sp.* as can be seen in Fig. 2.

DISCUSSION

The presence of CPS (*Staphylococcus aureus* and other species) in raw goat milk has been reported throughout the goat lactation period in all three seasons. The average results obtained in the spring months (1.4×10^3 CFU/ml) showed a low standard deviation (± 0.0957), while in the summer months the microbial population was quantified at 3.2×10^3 CFU/ml, with a standard deviation of ± 0.3806 and in autumn at a value of 2.8×10^3 CFU/ml, with a standard deviation of ± 0.1302.

The mean values of the number of coagulase-positive staphylococci recorded in spring, summer, and autumn for the raw goat milk samples were transformed to logarithm base 10, graphically representing the seasonal variation according to Fig. 3.

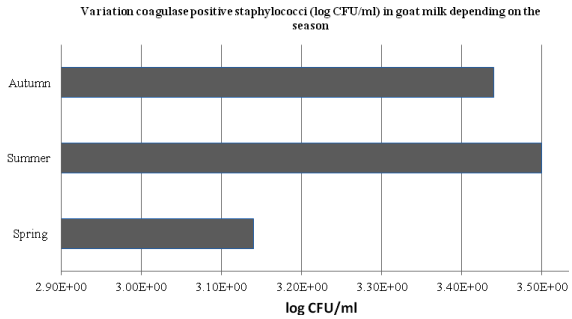

Figure 3. Logarithmic values of CFU/ml coagulase-positive staphylococci in goat milk depending on the season

The highest raw milk contamination rate was recorded in the summer months when the CPS number was of 3.5 log CFU/ml, followed by the autumn months when milk production decreased, having an average value of 3.44 log CFU/ml. In spring, when dairy production is high, the contamination of raw milk is of 3.14 log CFU / ml, lower in comparison with the raw milk obtained during summer and autumn. The high temperature of the milking environment has favored the multiplication of potentially pathogenic germs because the optimal environmental temperature for *Staphylococcus aureus* growth is between 25-40 °C [2].

Previous studies on the rate of CPS contamination of the raw goat milk showed a significant variation in the monthly average of coagulase-positive staphylococci from <1.69 log UFC / ml to 2.65 log CFU/ml [10]. The results obtained in the three milking seasons showed that the limit of 10^5 CFU/ml, meaning the number of CPS that can produce certain doses of enterotoxins, capable of inducing food poisoning [8, 12], was not exceeded. In previous studies, a minimum concentration of 10^6 UFC / ml was acceptable for S. aureus in order to perform assays on the presence of the staphylococcal enterotoxin in food [1, 5, 11, 14, 20].

In the case of the raw milk collected from goats bred in extensive system, contamination could have occurred during non-hygienic milking or by transmission of the pathogen through contaminated hands, but contamination with *Staphylococcus aureus* (for example) in raw milk may also emerge due to mammary glands infection [18, 23].

The presence of these bacteria (CPS) can cause udder infection [4, 9, 13, 15, 19, 22, 27], *Staphylococcus sp.* are the main agents of intramammary infections in small ruminants. The level of raw milk contamination with pathogenic bacteria may be increased when the mastitis affect in the flock.

The importance of determining the degree of raw milk contamination with Staphylococcus is strictly related to the quality of the obtained dairy products. On the other hand, fermentation and maturation of the cheese reduce the likelihood of producing enterotoxins

[29]. Knowing the degree of raw milk contamination with CPS and other species is a prerequisite for assessing the risks associated with these bacteria in milk and dairy products marketed to consumers by predictive microbiology.

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