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Yeasts in the Raw Ewe's Milk

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ABSTRACT

***Background*:** The contamination of milk by fungi, often represented by potentially pathogenic species, may pose a risk for consumers, when the product is consumed in natura or even in the processed form. Also, it should be considered detrimental effects in the processing of milk derivative products. In relationship to other milk producing animal species, ovine milk occupies the fourth place in the global production, contributing with 1.3% from the grand total. In Brazil, the majority of sheep's milk is used for the production of fine cheeses and yogurts. The aim of this study was to evaluate the occurrence of yeasts in the ewe's milk.

***Materials, Methods & Results*:** Five hundred and eighty-eight milk samples were obtained, of which 106 came from animals with clinical mastitis and 482 from healthy animals. Aliquots of 0.1 mL milk were first plated on acidified yeast medium agar. Then the yeast colonies were streaked onto Hicrome Candida Differential Agar Base (HIMEDIA®) and subjected to biochemical analysis by API 20C system (Biomérieux®). The identification was made by using conventional standardized test panel. A total of 60 fungal species were isolated from 53 (9%) milk samples. They were classified into the following genera: *Candida* spp. (70.00%), *Rhodotorula* spp. (11.70%), *Trichosporon* spp. (6.70%), *Geotrichum* spp. (5.00%), *Pichia* spp. (5.00%) and *Cryptococcus* sp. (1.70%). Potentially pathogenic yeasts were identified as *Candida glabrata* (n = 8), *C. tropicalis* (n = 6), *C. parapsilosis* 1 (n = 5), *C. albicans* (n = 4), *Pichia guilliermondii* (n = 3) and *Trichosporon asahii* (n = 1).

***Discussion*:** The majority of sheep's milk is not directly consumed by the population, being mainly destined for the production of cheeses of important market value. Therefore, typical sensorial and organoleptic characteristics found in cheese may be influenced, as early as the beginning of its production, by factors that interfere in the quantity and quality of milk, such as the milking technique used, udder infections and herd management. The research of filamentous fungi and yeasts is not routinely performed in raw milk of bovines or other species. However, some studies have already indicated the presence of a great diversity of fungal organisms originated from mastitis-free animals. The majority of the yeasts are considered saprotrophic, being found in storage tanks of milk from healthy animals, although they have been found, in some cases, in samples of milk from animals suffering from mastitis. Most studies on mycotic mastitis are associated with cows, where fungal agents are not considered primary. These agents are generally due to environmental contaminants associated with poor hygienic practices. In conclusion, the results stress the necessity to perform mycological test, for the correct diagnosis and monitoring of mastitis cases in ovine. There are few studies on the diversity of the fungal microbiota in the milk of small ruminants. The data presented herein corroborates results obtained in previous studies and also emphasize that consumption of ewe's milk and its products that have been contaminated with potentially pathogenic microorganisms constitute a direct risk to public health since many of these organisms are medically important yeasts and capable of causing infections, primarily in individuals with compromised immunity. Moreover, with regard to the production of dairy products made from sheep's milk, particularly cheese, microbiological contamination may affect the quality and shelf life of the final product, causing considerable economic losses.

Keywords: yeasts, ewe's milk, public health, microbiological contamination, mastitis.

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INTRODUCTION

In comparison with other milk-producing animal species, ovine milk occupies the fourth place in the global production, contributing with 1.3% from the grand total [7]. In Brazil, the majority of sheep's milk is used for the production of fine cheeses and yogurts [30].

The contamination of milk by fungi, often represented by potentially pathogenic species, may pose a risk for consumers, when the product is consumed *in natura* or even in the processed form. Additionally, negative effects should be also considered in the production of its derivatives, since alterations in the physical and organoleptic characteristics of milk may affect the quality and shelf life of dairy products [4,5,15].

In Brazil, researches carried out over the last years demonstrated the presence of several types of yeast in the milk of cows with or without mastitis [3,10,11,31,34]. There are few studies in the world literature on the diversity of the fungal microbiota in the milk of small ruminants. The objective of this study was to evaluate the occurrence of yeasts in the milk of healthy sheep and of those with clinical mastitis.

MATERIALS AND METHODS

This investigation was related to a study with 311 sheep of the Lacaune breed (72 of which had clinical mastitis and 239 were healthy), raised in a feedlot system located in the metropolitan area of Porto Alegre, State of Rio Grande do Sul (RS), southern Brazil, during the period of March/2010 to March/2012. The sheep did not receive an antibacterial treatment before the sampling was carried out.

Clinical mastitis was characterized through the presence of clinical signs in the mammary gland (pain, heat, redness) and an abnormal milk secretion. Sheep that did now show an apparent involvement of the mammary gland, whose macroscopic milk characteristics were normal and which presented negative reactions for the California Mastitis Test (CMT) were considered healthy.

A total of 588 sheep milk samples were obtained, of which 106 came from sheep with clinical mastitis and 482 from apparently healthy animals (without mastitis). The samples (10 mL) were aseptically collected into sterile flasks, after the appropriate asepsis of their tops using alcohol 70ºGL. The samples were immediately sent to the laboratory, inside polystyrene boxes containing ice, and then they were streaked onto different culture media.

Aliquots of 0.1 mL from the milk samples were streaked using the streak plate technique in triplicate, on acidified yeast medium agar (0.3% yeast extract, 0.3% malt extract, 1% glucose, 0.5% peptone, 2% agar, 400 mg/L chloramphenicol, pH 4.5) for the mycological analysis. The plates were incubated between 3 and 5 days at a temperature between 22ºC and 25ºC, for the isolation of both pathogenic and non-pathogenic yeasts. Samples that presented more than five morphologically identical colonies were considered positive. After this period, each morphological type was isolated and purified on Petri plates containing yeast medium agar. The isolates were then stored inside test tubes containing Sabouraud Dextrose Agar[1], covered in sterile mineral oil and kept in the refrigerator. Initially, the yeasts were characterized through physiological routine assays, according to tests routinely used [2,35], followed by differential tests, such as chlamydoconidia production and germ tube [24], and also through cultivation in Hicrome Candida Differential Agar Base[2]. The final identification of yeasts and yeast-like fungi was made through the API 20 C system[3]. Isolates producing arthroconidia were classified into the *Geotrichum* or *Trichosporon* genera.

Table 1. Mycological result of ewe's milk samples, collected in the period between March 2010 and March 2012, in the metropolitan area of Porto Alegre, RS, southern Brazil.

Number of Samples	Positive		Fungal Isolates
	N	(%)	
Healthy 482	31	(6,4%)	*Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *Candida* spp., *Cryptococcus laurentii*, *Geotrichum* spp., *Pichia guilliermondii*, *Rhodotorula* spp., *Trichosporon asahii*, *Trichosporon mucoides*, *Trichosporon* spp.
Mastitis 106	22	(20,7%)	*Candida albicans*, *C. glabrata*, *C. tropicalis*, *Pichia guilliermondii*
Total 588	53	(9%)	

RESULTS

A total of 60 fungal species were isolated from 53 (9.0%) milk samples (Table 1). They were classified into the following genera: *Candida* spp. (70.0%), *Rhodotorula* spp. (11.7%), *Trichosporon* spp. (6.7%), *Geotrichum* spp. (5.0%), *Pichia* spp. (5.0%) e *Cryptococcus* sp. (1.7%). Potentially pathogenic yeasts were identified as *Candida glabrata* (n = 8), *C. tropicalis* (n = 6), *C. parapsilosis* 1 (n = 5), *C. albicans* (n = 4), *Pichia guilliermondii* (n = 3) and *Trichosporon asahii* (n = 1).

DISCUSSION

The majority of sheep's milk is not directly consumed by the population, being mainly destined for the production of cheeses of important market value. Therefore, typical sensorial and organoleptic characteristics found in cheese may be influenced, as early as the beginning of its production, by factors that interfere in the quantity and quality of milk, such as the milking technique used, udder infections and herd management [25].

The research of filamentous fungi and yeasts is not routinely performed in raw milk of bovines or other species. However, some studies have already indicated the presence of a great diversity of fungal organisms originated from mastitis-free animals. In Rio Grande do Sul, a study made with 36 raw milk samples detected 80 isolates belonging to the *Kluyveromyces*, *Rhodotorula*, *Candida*, *Geotrichum* and *Trichosporon* genera [32]. In the same State, another study that used 15 in natura samples of goat's milk detected the presence of 56 yeasts, identified as belonging to the *Bullera*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Dekkera*, *Pichia*, *Rhodotorula*, *Sporodiobolus*, *Trichosporon*, *Yarrowia* and *Zygoascus* genera [33]. In Italy, analyses made with in natura buffalo milk isolated the Candida, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Trichosporon* and *Yarrowia* genera [9]; the same study was performed with in natura sheep's milk, and the genera *Bullera*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Rhodotorula*, *Trichosporon*,*Yarrowia* and *Kluyveromyces* were present. In the latter case, the contamination level was very high, in comparison with other normal milk samples from goat, cows and buffaloes. The results observed in previous studies were similar to those found in this study, where the most prevailing genera were *Candida*, *Cryptococcus*, *Geotrichum*, *Pichia*, *Rhodotorula* and *Trichosporon*.

The majority of the yeasts are considered saprotrophic, being found in storage tanks of milk from healthy animals [22], although they have been found, in some cases, in samples of milk from animals suffering from mastitis [6,19,21,27,28]. Most studies on mycotic mastitis are associated with cows, where fungal agents are not considered primary. These agents are generally due to environmental contaminants associated with poor hygienic practices [32].

In Rio Grande do Sul, only a few studies will focus on the prevalence of mycotic mastitis in milk-producing animals. In one investigation, *Candida* spp. were isolated in 1.3% of 896 milk samples analyzed from animals suffering from mastitis, among which 0.9% contained Candida albicans [14]. In a different study performed in the same State, *C. albicans* was absent from milk of animals with clinical and subclinical mastitis, although yeasts from the *Candida* genera represented 37.9% of the total amount of isolates [34]. In Minas Gerais, a study with 1710 samples of milk from animals suffering from mastitis detected the presence of 56 yeasts, and *C. albicans* was the prevailing species, representing 28.1% of the isolates [11]. Additionally, in another study made in Brasil, *C. albicans* was isolated in 8.9% of 260 samples from cows suffering from mastitis [27]. In the current study, *C. albicans* was isolated in 6.7% of 588 milk samples from healthy animals and from those suffering from mastitis.

In Egipt, the presence of yeasts was detected in 4.84% of 196 samples of milk from ewe with subclinical mastitis [23]. In a study made in Iraq with 140 samples from ewe suffering from mastitis, the contamination in 7.9% of them was attributed to fungi in general, among which only three yeast genera were identified: *Candida*, *Cryptococcus* and *Saccharomyces* [1]. Additionally, studies made in Argentina and Poland with milk from animals suffering from mastitis revealed the single isolation of a small amount of *Candida* spp. [16,20]. In the present investigation, the presence of yeasts and yeast-like fungi was detected in 9% (53) of the total samples. Only the species *Candida albicans*, *C. glabrata*, *C. tropicalis* and *Pichia guilliermondii* were found both in normal and mastitic milk samples, whereas the others species were isolated from normal milk only.

The results of this study revealed that only 1.7% of the isolates were originated from yeasts of

the *Cryptococcus* genus. Although *Cryptococcus neoformans* is the most frequent species involved in mastitis cases [12,13], *Cryptococcus* sp., *C. laurentii* and *C. curvatus* have also already been associated with mastitis in cows [10,17,18], sheep [29], buffaloes [26] and goats [8].

CONCLUSION

In conclusion, the results stress the necessity to perform mycological test, for the correct diagnosis and monitoring of mastitis cases in ovine. The consumption of ewe's milk and its products that have been contaminated with potentially pathogenic microorganisms constitute a direct risk to public health since many of these organisms are medically important yeasts and capable of causing infections, primarily in individuals with compromised immunity. Moreover, with regard to the production of dairy products made from sheep's milk, particularly cheese, microbiological contamination may affect the quality and shelf life of the final product, causing considerable economic losses.

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