Mycopathologia (2015) 179:327–331
DOI 10.1007/s11046-014-9845-2

Survey of Bovine Mycotic Mastitis in Different Mammary Gland Statuses in Two North-Eastern Regions of Algeria

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Received: 7 June 2014 / Accepted: 30 November 2014 / Published online: 7 December 2014

Abstract The aim of this study was to evaluate the prevalence of mycotic mastitis in different mammary gland statuses. The study was conducted on 304 dairy cows from ten farms in two north-eastern regions in Algeria; Guelma and Souk Ahras with 922 and 199 samples, respectively, forming thus a total number of 1,121 milk samples. A total of 321 milk samples were collected from clinical mastitis, 544 milk samples from subclinical mastitis and 256 milk samples from healthy mammary glands. Mycological analyses revealed that 10.17 % of the treated samples were positive recording 114 species of fungi including 88 yeasts and 26 moulds. The most frequent species was *Candida kefyr* followed by *C. albicans*, *C. guilliermondii*, *C. famata*, *C. tropicalis*, *C. colliculosa*, *C. krusei*, *C. rugosa*, *C. glabrata*, *C. parapsilosis*, *C. inconspicua*, *Trichosporon* sp., *Rhodotorula glutinis* and *Saccharomyces fragilis*. Mould species have also been isolated from samples of both healthy milk and clinical mastitis milk. *Aspergillus amstelodami* (from glaucus group), *A. fumigatus* and *Geotrichum candidum* were identified, while the other species including *Penicillium* sp. and *Cladosporium* sp. were not identified.

Keywords Candida · Mammary gland · Mastitis · Milk · Yeast

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Introduction

Mastitis is a mammary gland inflammation that results from a biological conflict between the existing microorganisms and the animal's udder. The economic consequences of this disease are very important. Besides the decrease in production, mastitis causes biochemical changes in milk [1]. In the majority of mastitis cases, bacteria are the etiological agents while mycotic mastitis represents only a small portion [2]. Since Rolle's princeps observation of mycotic mastitis [3], several species of fungi were isolated. A predominance of the genus *Candida* was recorded in most studies and surveys [4–10]. In Algeria, no data are available about the existence of mycotic mastitis. Indeed, the objectives of this study are to identify the species of fungi, to determine their isolation frequencies and the prevalence of mycotic

mastitis in dairy cows in two north-eastern regions in Algeria and finally to assess their impact on economy and on public health.

Materials and Methods

The study was conducted on 304 dairy cows of different breeds, ameliorated ones (Holstein and Montbeliard) and local ones (Guelmoise and Cheurfa). A total of 1,121 samples were collected during 23 months from July 2010 to May 2012. The selected dairy cows are from ten farms in two north-eastern regions in Algeria; Guelma (six municipalities) and Souk Ahras (two municipalities) with 922 and 199 samples, respectively. Each sample corresponds to a quarter of a different animal. In this study, samples were collected in different mammary glands conditions. Actually, there were 256 milk samples from healthy mammary glands and 544 milk samples from cows with subclinical mastitis detected by California Mastitis Test, Schalm and Noorlander [11] and Schneider et al. [12]. The CMT score included in the study is higher than or equal to 1. Finally, 321 milk samples from cows suffering from clinical mastitis diagnosed after a general check-up of cows and a special examination of the mammary gland.

The samples were taken after udder-washing with bleach and disinfection with alcohol at 70°. Samples of 14 ml of milk were put in sterile tubes, immediately closed and placed in isothermal cooler at 4 °C. Milk samples were centrifuged at 4,000 rpm for 5 min [8], and then, a direct examination of a pellet drop was essential for the detection of fungal elements, yeast, pseudohyphae and hyphae. 0.5 ml of the milk pellet was homogenized in 4.5 ml of Sabouraud broth (Fleuka^{TM}) at a pH of 3.7 and incubated at 37 °C [13]. After 10 days of incubation, 50 µL of the culture was inoculated in Sabouraud chloramphenicol agar (bioMérieux^{TM}), and then, the dishes were incubated at 37 °C for 5 days.

Fungi isolated in this study were identified based on the observed morphological characteristics. Chlamydoconidium, pseudohyphae and hyphae are screened on Rice cream medium (Pasteur Institute of Algiers). Blood serum was used to detect germinative tubes. *Cryptococcus* capsules were observed after being stained with India ink. Biochemical characteristics taken into account include: the sensitivity of fungi to Cycloheximide 0.1 % (Pasteur Institute of Algiers), urea hydrolysis and the auxanographic characters (the system API 20 C Aux). The identification of yeast species was performed by apiweb software (bio-Merieux^{TM}) and the key described by Kurtzman and Fell [13], while mould identification was based on the key of Botton et al. [14].

Results

The mycological analyses showed that 10.17 % of the examined samples were positive (Table 1), 89/922 in Guelma and 25/199 in Souk Ahras. Fungi were detected in 12.50 % of the healthy milk samples, in 5.88 % of subclinical mastitis milk samples and in 15.57 % of clinical mastitis milk samples. A variety of fungal species are recorded in the three categories of milk. We also noted a predominance of *Candida* yeast with 71.93 % that represents eleven species, especially *Candida kefyr* which is the most frequent one with 16.67 % isolates, followed by *Candida albicans* with 13.16 % isolates. In moulds species, *Aspergillus* was the most frequent one with a rate of 14.92 %.

In 256 milk samples from healthy mammary glands, only 32 were positive. We recorded the prevalence of yeasts with 17/32 isolates, especially the genus *Candida* with 16/32 isolates and *C. kefyr* with 4/32 isolates. Moulds isolated from this milk samples represent 15/32 of the isolates.

Only 32 isolates from 544 subclinical mastitis milk samples were recorded. We have noticed the predominance of *Candida* yeast with 30/32 isolates represented by nine different species. The identification revealed a co-dominance of *C. famata* and *C. kefyr* with 6/32 isolates.

In 321 milk samples of clinical mastitis, 50 isolates were recorded. The three quarters of the fungi species were yeasts of the genus *Candida* with eight different species. The most frequently isolated species were *C. albicans* with 12/50 isolates. In mould species, *Aspergillus fumigatus* was relatively frequent with 6/50 isolates.

Discussion

Many studies conducted in different countries on mycotic mastitis revealed that the frequency of fungi

Table 1 The genera and species of yeasts isolated from cases of bovine mastitis in different mammary gland statuses

Genera	Species	Mastitis status of mammary glands			Frequency isolation	
		Healthy	Subclinical	Clinical	Nombre	%
Candida	*C. kefyr*	4	6	9	19	16.67
	C. albicans	0	3	12	15	13.16
	C. guilliermondii	3	4	7	14	12.28
	C. famata	3	6	3	12	10.53
	C. tropicalis	2	3	2	7	6.14
	C. colliculosa	0	4	0	4	3.51
	C. krusei	1	1	1	3	2.63
	C. rugosa	0	1	1	2	1.75
	C. glabrata	1	0	1	2	1.75
	C. parapsilosis	2	0	0	2	1.75
	C. inconspicua	0	2	0	2	1.75
		(16)[a]	(30)[a]	(36)[a]	(82)[a]	(71.93)[b]
Trichosporon	Not identified	1	1	1	3	2.63
Rhodotorula	*R. glutinis*	0	1	1	2	1.75
Saccharomyces	*S. fragilis*	0	0	1	1	0.88
Aspergillus	*A. amstelodami*	8	0	1	9	7.90
	A. fumigatus	2	0	6	8	7.02
		(10)[a]	(0)[a]	(7)[a]	(17)[a]	(14.92)[b]
Penicillium sp.	Not identified	3	0	4	7	6.14
Geotrichum	*G. candidum*	1	0	0	1	0.88
Cladosporium sp.	Not identified	1	0	0	1	0.88
Total		32/256 (12.50 %)	32/544 (5.88 %)	50/321 (15.57 %)	114/1,121 (10.17 %)	100

[a] The sum of the species in a genus

[b] Percentage rate of the genus

isolation is continuously changing. Zaragoza et al. [10] reported a rate of 25.75 % in Mexico, a rate of 6.1 % was reported in Egypt [15], 1.3 % in Denmark [16] and South Korea [17] and 9.6 % in Poland [9]. Moreover, this variability is also observed in the same country; in Brazil, isolation rates of 12.07, 25.40 and 27.42 % were recorded, respectively, by Costa et al. [18] Casia Moacir Dos Santos and Marin [19] and Spanamberg et al. [20]. In our study, fungi isolation frequency in all milk samples collected in three mammary gland statuses was at the rate of 10.17 %.

We recorded 88 yeast isolates against 26 mould isolates. Yeast abundance may be explained by their pre-existence in the mammary glands. The pre-existing yeasts can be disrupted by several factors such as antibiotherapy and corticotherapy that are highly recommended in mastitis for curative or prophylactic purpose [21]. According to Mehnert et al. [22], some yeast species use antibiotics as a nitrogen source for growth, so this can explain their abundance.

In our study, the fungi species isolated from milk collected from cows with clinical and subclinical mastitis are considered pathogens. The pathogeneity of these fungi results in an inflammation of the mammary gland. In addition, a high fungal density found after the inoculation on appropriate media proves the infection. On the other hand, the absence of clinical signs and the low CMT score (equal to 0) observed in the milk of healthy mammary glands lead us to think that the fungi species found in this category of milk are non-pathogenic fungi or saprophytes of the mammary gland.

According to the results of mycological analysis, we noticed that *Candida kefyr* was relatively frequent. Lagneau et al. [8] have found the same results in a similar study performed in Belgium. In Poland,

Krukowski et al. [9] reported a predominance of this species in their studies on the prevalence of mycotic mastitis. *Candida kefyr* was also isolated in the mastitis milk samples by several authors, Aalbaek et al. [16], Guilhon et al. [3], Pengov [23], McDonald et al. [24] and Richard et al. [7].

In our study, *C. kefyr* isolation frequency was higher in the milk samples of subclinical and clinical mastitis than its isolation frequency in healthy milk samples. Indeed, since its first observation in France by Guilhon et al. [3], the pathogeny of this yeast is well known and demonstrated experimentally by Aalbaek et al. [16]. It can grow at temperatures around 42 °C [8]. This represents a necessary condition to infect the mammary gland. *C. kefyr* is among the Candida species that assimilate lactose [25], which is a crucial character for the growth in milk medium. According to Guilhon et al. [3], the source of *Candida kefyr* is the cattle faeces that are perpetually present in the mammary glands.

Candida albicans is the second most frequent species recorded in our study. This yeast has been isolated only in milk samples of clinical and subclinical mastitis. Similar results are mentioned in a recent study by Zaragoza et al. [10]. The absence of *Candida albicans* in the healthy milk samples can be explained by its pathogeny in mammary glands. It causes an inflammatory reaction resulting from the increase of the CMT score. Indeed, this yeast was isolated by several authors [4–7, 16, 19, 23].

In our study, *C. guilliermondii*, *C. famata*, *C. tropicalis* and *C. Krusei* are isolated from the three categories of milk samples. Several studies reported that these Candida species were often found in the milk samples of clinical and subclinical mastitis, and even in healthy milk samples [8, 10, 18, 26].

Yet, *Candida rugosa* was isolated from the milk samples of clinical and subclinical mastitis. A similar result was recently declared by Şeker in Turkey [26].

However, *C. glabrata* was only isolated from the samples of healthy milk and clinical mastitis milk samples. Its absence in the milk samples of subclinical mastitis can be explained by the presence of many bacteria producing metabolites that act as antagonists and inhibitors of yeast growth. Moreover, these bacteria compete for substrates in milk medium [27].

Candida colliculosa is an ascomycete yeast with a great ability to ferment sugars. It is widely used in the production of white beer and fermented drinks [28]. In our study, *Candida colliculosa* was not isolated from the milk samples of mastitis. This species, which is rarely isolated in diseases, was reported for the first time in a hemoculture from a patient with a gastrointestinal cancer. It is also found worldwide in many foods, mainly fermented ones. *Candida colliculosa* was also isolated from the liver and the spleen of wild rodents in Venezuela. In 1954, *Candida colliculosa* was isolated from a skin lesion in a child of 3 months in Brazil [28]. Recently, this yeast was isolated from a fungal endocarditis in a man of 71 years [29]. This patient was interested in gardening and in the production of his own dairy products. According to these authors, the consumption of dairy products may be a risk factor for the infection by *Candida colliculosa*. The extremely rare pathogenicity of *Candida colliculosa* can be explained by the results of Dworecka-Kaszak et al. [30]. In their study about yeasts isolated on mastitis and identified by PCR, they indicate that strains of fungi phenotypically classified as the same Candida species can have different genotypes.

According to our results, *C. parapsilosis* and *C. inconspicua* can be considered as opportunistic pathogens, because they are detected only in healthy milk samples and subclinical mastitis milk samples. On the other hand, *C. parapsilosis* is reported as a pathogen responsible for clinical mastitis in several studies [9, 10, 18, 19, 30].

Numerous authors reported the presence of *Trichosporon* sp. [18, 31], *Rhodotorula glutinis* [9, 18, 20] and *Saccharomyces fragilis* [3] in mycotic mastitis. In the present study, these species were also recorded, especially in clinical and subclinical mastitis cases.

Concerning the isolated mould species, we have noted their exclusive presence in the milk samples of clinical mastitis and especially in the healthy milk samples. They may represent just contaminations, but this is not confirmed or proved because many studies about mycotic mastitis reported their presence [4, 6, 18, 31–33], except for *Cladosporium* sp. This species has not been considered as a mastitis agent. However, it was frequently isolated as a laboratory contaminant [34, 35].

The detection of healthy carriers in the studied dairy cows was significant with a rate of 12.5 %. In the most studied farms, milk is intended for human consumption. This can be considered as a danger for public health. The direct fungi transmission from milk to man has never really been proven, although it cannot be completely excluded. Besides, yeasts resistance is important; they were found viable in butter

and cheese made from the milk of sick cows, even after more than 7 years [36]. Therefore, to prevent any risk of human or animal infection, it is essential to diagnose subclinical mastitis milk, especially in the healthy carriers, to avoid their direct consumption or their use in dairy products. This illustrates the economic consequences of this disease.

Acknowledgments The authors are particularly grateful for assistance from Dr. Bentourki Aymen.

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