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Survey of bovine mycotic mastitis in dairy herds in the State of São Paulo, Brazil

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Abstract. The purpose of this study was to isolate fungi from the quarter milk of cow udders from several dairy herds and to identify the different genera and species involved in mastitis. A total of 2078 milk samples from normal, clinical and subclinical mastitis quarters from 22 dairy herds of 16 districts in the State of São Paulo, Brazil was utilized in this survey. Two hundred and fifty one (12.07%) fungi were isolated from the samples. Two hundred and eight of these (82.86%) were yeasts and 30 (11.95%) were moulds. The fungi were isolated in pure culture (24.77%) or in cultures mixed with bacteria (72.22%). The yeasts isolated were: *Cryptococcus* spp. (71 strains), *Rhodotorula* spp. (40), *Candida* spp. (68), *Trichosporon cutaneum* (21), *Aureobasidium pullulans* (7), and *Pichia ohmeri* (1). Moulds classified in following genera were also isolated: *Aspergillus* (3), *Penicillium* (3), *Alternaria* (3), *Phoma* (3), *Epicoccum* (2), and *Geotrichum* (16).

Key words: Bovine mastitis, Mammary gland, *Aspergillus* spp., *Candida* spp., *Cryptococcus* spp., *Penicillium* spp., *Rhodotorula* spp., *Trichosporon* sp.

Introduction

Mastitis is the most important health problem in bovine dairy herds. Fernandes [1] stated, that Fleischer in 1930 was the first to describe a case of mycotic mastitis. Since then, fungi have been reported in many other countries as causative agents of mastitis. This was well reviewed by Giesecke et al. [2], where the authors listed the species of fungi isolated in bovine mastitis and their literature references.

The first description of mycotic mastitis by Brazilian authors dates from 1976, when Minami et al. [3] described clinical mastitis caused by *Cryptococcus laurentii* in a cow. Following this report two other mycotic mastitis occurrences were reported in Brazil by Mos et al. in 1978 [4]. They isolated *Candida lusitanae* from a Holstein–Frisian cow with clinical mastitis.

The purpose of our study was to isolate fungi from the mammary glands of cows from several dairy herds and to identify the different genera and species involved which could lead to economic losses and public health hazards.

Material and methods

A total of 2078 milk samples from clinical and subclinical mastitis, detected by the California Mastitis Test, Schalm & Noorlander [5] and a modified Whiteside test, Murphy & Hanson [6] from 22 dairy herds of 16 counties of São Paulo State, Brazil, were examined. Approximately

10% of this total represented normal milk samples, they were analyzed to detect carriers.

These milk samples were always aseptically collected by the same persons that conducted the laboratory work. They were kept at as a temperature of 0–4 °C, and plated as soon as possible on brain-heart-infusion agar (BHI)[1], mannitol salt agar[1], aescolin-tallium acetate-crystal violet blood agar, and Sabouraud dextrose agar[1] with 100 mg chloromycetin.

The plates for bacterial isolation were incubated for 72 h at 37 °C and the Sabouraud dextrose agar plates were kept at 25 °C for at least a month before being discarded.

The isolated bacteria were identified according to Lennette et al. [7] and classified according to Bergey's Manual of Systematic Bacteriology [8, 9] using morphological, cultural and biochemical characteristics.

Yeast identification was performed according to Lodder [10], Barnett et al. [11] and Kreger-van Rij [12]. Mould identification was based on the keys of Barnet & Hunter [13] and Arx [14].

Results

From the 2078 milk samples examined 251 fungi were isolated (12.07%). Among these 208 (82.86%) were yeasts and 30 (11.95%) were moulds (Table 1). These 208 yeast isolates were classified among 7 genera. The most frequently isolated were members of the genus *Cryptococcus* (*C. albidus*, *C. flavus*, *C. laurentii* and *C. luteolus*); the second most frequently isolated yeasts were *Candida* spp. (*C. albicans*, *C. catenulata*, *C. ciferri*, *C. freyschusii*, *C. famata*, *C. glabrosa*, *C. globosa*, *C. guilliermondii*, *C. haemulonii*, *C. intermedia*, *C. krusei*, *C. magii*, *C. parapsilosis*, *C. rugosa*, *C. sheatae*, *C. sorbosa*, *C. tenuis*, *C. tropicalis*, *C. zeylanoides*, *C. variabilis*). The genera and species of fungi isolated are presented in Table 1. It was verified that the frequency of isolation was as low as 0.00% in some herds. These results are remarkable, since fungi are ubiquitous in the environment and the lack of isolation of these microrganisms in at least four herds located in four different counties confirms that the adopted methodology for sample collection was effective. Among the 251 fungi isolated, 44.80% were associated with quarters with clinical mastitis, and 18.40% to quarters with subclinical mastitis. The occurrence of 31.60% carrier quarters (normal quarters harbouring fungi) was documented.

Pure fungal isolates were obtained in 24.77% of the cultures, the remaining 75.22% were mixed with bacteria. The bacteria were primarily *Staphylococcus* spp., *Streptococcus* and *Corynebacterium* (*C. bovis*) spp.

Table 2 shows the relationship between yeast isolates and the intensity of the inflamatory reaction of the mammary glands, to their presence. This evaluation was based on clinical signs and the CMT and Whiteside tests. It can be noted in Table 2 that fungi in the genera *Candida* and *Cryptococcus* were correlated predominantly with clinical severity (57.35%, 56.33%, respectively). In Table 2 the percentage distribution of mould genera was not included because they occurred in such reduced numbers that it might lead to wrong conclusions.

Discussion

The percentage of fungal isolations in surveys carried out in many countries varies considerably, thus, Awad et al. [15] registered 6.1% isolation in Egypt; Yeo & Choi [16] registered 1.3% in a Korean survey; Giesecke et al. [17] in South Africa registered 12.12% of yeasts isolated from bovine quarters; Bellani et al. [18] registered 4.4% in Italy.

In the present study there was registered an occurrence of 12.07% of fungi (Table 1). The survey was conducted, as described previously, in 22 dairy herds in 18 counties of the state of São Paulo, Brazil, great differences among the fungal isolation percentages in the counties were observed, ranging from 0.00% to 73.03%. The

Table 1. Bovine mycotic mastitis. The genera and species of fungi isolated from 2078 milk samples from 22 dairy herds in 16 districts of the state of São Paulo, Brazil

Number	Genera	Species
71	*Cryptococcus*	*C. albidus*, *C. flavus*; *C. laurentii*, *C. luteolos*
68	*Candida*	*C. albicans*; *C. catenulata*; *C. ciferri*; *C. freyschusii* *C. famata*; *C. glabrosa*; *C. globosa*; *C. guilliermondii*; *C. haemuloni*; *C. intermedia*; *C. krusei*; *C. mogii*; *C. parapsilosis*; *C. rugosa*; *C. shehatae*; *C. sorbosa*; *C. tenuisis*; *C. tropicalis* *C. zeylanoides*; *C. variabilis*
40	*Rhodotorula*	*R. glutinas*, *R. minuta*; *R. Rubra*
21	*Trichosporon*	*T. cutaneum*
7	*Aureobasidium*	*A. pullulans*
1	*Pichia*	*P. ohmeri*
16	*Geotricum*	*G. candidum*
3	*Aspergillus* spp.	not identified
3	*Penicillium* spp.	not identified
3	*Alternaria* spp.	not identified
3	*Phoma* spp.	not identified
2	*Epicoccum* spp.	not identified
Total	251	
	13 unidentified	(12.07%)

Table 2. Relationship of intensity of mammary glands inflammatory reaction and the main yeast isolates, considering the analysis of 2078 milk samples from 22 dairy herds of 16 districts, São Paulo State, Brazil

Genera	Intensity		
	Mastitis		
	Clinical %	Subclinical %	Carrier %
Candida spp.	57.35	20.58	22.05
Cryptococcus spp.	56.33	8.45	35.21
Rhodotorula spp.	40.00	22.50	37.50
Trichosporon spp.	33.33	40.90	22.72

marked differences may have been due to ecological and managerial variations in the herds.

The percentual occurrence of the yeasts was higher than moulds. In this study, 208 yeasts were isolated (82.86%) and only 30 moulds (11.95%). The common occurrence of yeasts, when compared to moulds, may be due to their better perpetuation in the mammary gland and also to the fact that some genera of yeasts can utilize antibiotics like penicillin and/or tetracycline as a nitrogen source for growth [19].

The identification of the yeasts yielded a variety of species (Table 1), some of which have previously been described as being pathogenic for the bovine mammary gland [15–17, 20, 21]. In this survey some species of yeasts that have not yet been isolated from bovine mastitis milk such as *Candida ciferri*, *C. shehatas*, *C. mugii*, *C. glabrosa*, *C. freyschyssii*, *C. haemulonii*, *C. variabilis*, *Cryptococcus flavus* and *C. luteolus*. These findings denote a dynamic relationship in the etiology of this process suggesting the pathogenic potential of these newly encountered species.

The high prevalence of *Cryptococcus* spp. detected in this study was also observed by Giesecke et al. [17] in 1968, but it differed from the results obtained by Richard et al. [21] in 1980 and Kadic et al. [22] in 1983, where *Candida* spp. were more prevalent.

Considering the moulds isolated (Table 1), this was the first recording of *Epicoccum* spp. and

Phoma spp. associated with bovine mastitis. They were isolated in pure culture. This is particularly important considering the public health implication since *Phoma* spp. and *Epicoccum* spp. are mycotoxin producers [23–24].

Fungi were isolated in pure culture or mixed with bacteria. Many of those obtained in pure growth were isolated from clinical mastitis quarter, showing their pathogenicity to the mammary gland being either primary or secondary disease agents. Even when associated with bacteria, they constitute a real problem once the therapy against bacterial mastitis is unsuccessful in eliminating the fungi present in the quarter. Conditions like the chronic form of mastitis, frequently subjected to prolonged, excessive and repeated antibacterial therapy, helps the establishment of fungal or mixed infections (bacteria and fungi), represent without doubt the most important predisposing cause of mycotic mastitis. The isolation of fungi, post-antibiotic treatments, suggests that they are not only resistant to them but can more readily produce disease in the absence of the rapidly multiplying bacterial competitors. According to Kauker [25], large doses of antibiotics cause a reduction in the vitamin A content in cattle, leading to injury to the udder's epithelium, thus facilitating the invasion of fungi. Many outbreaks of mycotic mastitis have occurred following intramammary antibiotic therapy as reported by Hulse [2], Galli [26], and Bolck et al. [27].

Conclusion

A level of 12.07% of mycotic mastitis is significant, considering the economic losses represented by the decrease in milk production, loss of the use of affected quarters, and sometimes of the affected cows as well as therapy costs.

It must be pointed out the public health hazard presented by the consumption of fungal contaminated milk because these microorganisms, are sometimes resistant to pasteurization, as for example, several species of *Aspergillus* [28]. Besides the potential risk presented by the fungi themselves, the deleterious effects of the toxins produced by some species of *Aspergillus*, *Penicillium*, *Epicoccum* and *Phoma*, must be taken into consideration.

Therefore, it can be concluded, that there is a need to call attention to the increasing participation of fungi in the etiology of bovine mastitis and that it deserves to be subjected to more detailed studies.

Dedication

This paper is dedicated as 'a memorial' to Prof. Dr. Rolando Cury for his professional example and advice.

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