Mycopathologia (2005)159: 251–253

Isolation of *Candida* spp. from mastitic bovine milk in Brazil

Rita de Casia dos Santos[1] & José Moacir Marin[2]
[1]*Departamento de Microbiologia da Faculdade de Ciências Agrária e Veterinárias de Jaboticabal, Universidade Estadual Paulista;* [2]*Departamento de Morfologia, Estomatologia e Fisiologia, FORP, Universidade de São Paulo*

Received 22 October 2003; accepted in revised form 4 June 2004

Abstract

The purpose of this study was to isolate yeast (*Candida*) from the quarter milk of cow udders from 37 dairy farms in Brazil and to identify the different species involved in mastitis. The samples were collected between October 2002 and February 2003. Two-hundred-and-sixty milk samples from cows with clinical and subclinical mastitis were examined. Milk samples were plated onto Blood agar, Mac Conkey agar and Sabouraud dextrose agar. Forty-five (17.3%) samples were positive for the genus *Candida*. The *Candida* species isolated were *C. krusei* (44.5%), *C. rugosa* (24.5%), *C. albicans* (8.9%), *C. guilliermondii* (8.9%), and others (13.2%). We also isolated *Escherichia coli* (26.5%), coagulase-positive *Staphylococcus* (25.0%), *Streptococcus* spp. (8.1%), *Enterobacter* spp. (8.1%), and other fungi (8.1%), among others.

Key words: cattle, mastitis, milk, yeast

Introduction

Bovine mastitis has been defined as an inflammation of the mammary gland usually as a consequence of microbial infection. A wide variety of microorganisms have been implicated as causative agents of bovine mastitis, which is the most important disease affecting dairy cows, and yeast infections contribute to the importance of the problem [1].

Yeast and fungi are normal flora of the soil and may colonize udder skin in small numbers [2]. They are considered to be opportunists [3] and produce disease when natural defense mechanisms are lowered.

The incidence of mastitis due to fungi is usually very low in dairy herds in the United States. Kirk and Bartlett [4] mention a prevalence of fungal mastitis of 2.0 to 7.0%. In tropical countries the percentage can be more elevated [5]. The most frequent organisms implicated are yeasts, especially of the genus *Candida* a few species of which have been recovered from infected glands.

The aim of the present study was to isolate and identify *Candida* from the mammary glands of cows with clinical and subclinical mastitis.

Materials and methods

Two-hundred-and-sixty milk samples from quartes with clinical and subclinical mastitis were collected between October 2002 and February 2003 from 37 dairy herds in Brazil.

The milk samples were always aseptically collected and plated onto Blood agar, Mac Conkey agar and Sabouraud dextrose agar. The plates were incubated at 37 °C for 72 h.

The isolated bacteria were identified according to Carter and Cole [6] and classified according to Bergey's Manual of Systematic Bacteriology [7] using morphological and biochemical characteristics. Yeast identification was performed according to Richard et al. [2]. Kreger-Van Rij [8] and Barnett et al. [9].

Results

Data concerning the etiology of bovine mastitis for 37 dairy herds in Brazil are presented in Table 1. *Escherichia coli* (26.5%), coagulase-positive *Staphylococcus* (25.0%), *Candida* spp. (17.3%), *Streptococcus* spp. (8.1%), *Enterobacter* spp. (8.1%) and other fungi (8.1%), as well as other microorganisms (6.9%) were isolated from 260 milk samples.

Forty-five milk samples were positive for *Candida* spp. (17.3%). The species of *Candida* isolated from cows with mastitis are presented in Table 2, and the most frequently isolated ones were *C. krusei* (44.5%), *C. rugosa* (24.5%), *C. albicans* (8.9%) and *C. guilliermondii* (8.9%).

Discussion

Yeast is a microorganism which may be found on a wide variety of substrates such as soil, plants and water. Most of these organisms are opportunists and sources of infection include the skin of the udder, udder secretion, milker's hands, milking machines, treatment instruments, floor, straw, feed, dust, soil, drug mixtures and sanitizing solutions [2].

Table 1. Etiological agents of bovine mastitis in 37 dairy herds in Brazil

Etiological agent	Number	%
Echerichia coli	69	26.5
Coagulase-positive *Staphylococcus*	65	25.0
Candida spp.	45	17.3
Streptococcus spp.	21	8.1
Enterobacter spp.	21	8.1
***Other fungi*	21	8.1
Proteus spp.	5	1.9
Pseudomonas spp.	3	1.1
**GNB*	3	1.1
Shigella spp.	2	0.8
Serratia spp.	1	0.4
Bacillus spp.	1	0.4
Edwardsiela spp.	1	0.4
Sarcina spp.	1	0.4
Providencia spp.	1	0.4
Total	260	100

* GNB-Gram Negative Bacillus.
** Including filamentous fungi and other yeasts.

Table 2. Candida species isolated from cases of bovine mastitis in 37 dairy herds

Species	Number	%
C krusei	20	44.5
C. rugosa	11	24.5
C. albicans	4	8.9
C. guilliermondii	4	8.9
C. parapsilosis	2	4.4
C. zeulanoide	1	2.2
C. vini	1	2.2
C. famata	1	2.2
C. tropicalis	1	2.2
Total	45	100

Candida is commonly viewed as an opportunistic yeast pathogen. On the normal host, the yeast has evolved to become a successful commensal. It expresses variant traits critical for existence on mucosal surfaces. In the abnormal host, the same traits become virulence characteristics accounting for invasive abilities as the delicate balance of *Candida* with the host shifts in favor of the yeast [10].

The percentage of fungal isolation in surveys carried out in many countries varies considerably, with 6.1% rates reported in Egypt [11], 1.3% in South Korea [12],1.3% in Denmark [13], 9.6% in Poland[14], and 12.07% in Brazil [5]. In the present study the percentage of fungal isolation was 25.4%, with 17.3% of the fungi being *Candida* spp. This rate of *Candida* spp. isolation (17.3%) was higher than the rate of isolation of molds plus other yeast (8.1%) (Table 1), in agreement with data reported by Costa et al. [5].

The yeast were isolated in pure culture or mixed with bacteria, as pure culture it was isolated in 32.0% of samples similar results were obtained by Costa et al. [5] and Krukowski et al. [14].

The species *C. krusei* accounted for more than one third of the strains isolated (Table 2). *C.krusei* was also the predominant species demonstrated by Farnsworth and Sorensen [15] and by Aalbaek et al. [13]. In several surveys of mycotic mastitis, *C. krusei*, *C. rugosa* and *C. albicans* were often demonstrated [2, 5, 13, 14, 15, 16].

Lagneau et al. [17] mention that an important pathogenic characteristic of mastitic yeast isolates is their ability to grow at temperatures above 40 °C. Note that the four species more frequently isolated in this study showed this ability.

Fungal mastitis has been reported to occur mostly after antibiotic treatment, often without microbiological examination of milk from affected mammary glands or after infusion of antibiotics that were often homemade [2, 18].

Therefore it should be concluded that fungal mastitis will become an increasing problem due to extensive use of nonspecific antibiotics for the treatment of mastitis.

Acknowledgements

The authors are grateful to Laboratório VITA-FORT Ltda for providing the mastitic milk samples.

References

1. Watts JL. Etiological agents of bovine mastitis. Vet Microbiol 1988; 16: 41–66.
2. Richard JL, McDonald DVM, Fichtner RE, Anderson AJ. Identification of yeasts from infected bovine mammary glands and their experimental infectivity in cattle. Am J Vet Res 1980; 12: 1991–1994.
3. Loftsgaard G, Lindquist K. Bovine mycotic mastitis. Acta Vet Scand 1960; 1: 201–220.
4. Kirk JH, Bartlett PC. Bovine mycotic mastitis.Comp Food Anim 1986; 8: 106–110.
5. Costa EO, Gandra CR, Pires MF, Coutinho SD, Teixeira CM. Survey of bovine mycotic mastitis in dairy herds in the state of São Paulo, Brazil. Mycopathologia 1993; 124: 13–17.
6. Carter GR, Cole JR Jr. Diagnostic Procedures in Veterinary Bacteriology and Mycology. New York: Academic Press, 1990; 620 pp.
7. Bergey's. Manual of Determinative Bacteriology, 9th. edn. Baltimore, USA: Willians and Wilkins, 1994.
8. Kreger-Van Rij NJW. The Yeast: A Taxonomic Study. Amsterdam, Elsevier 1984: 1082 pp.
9. Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and Identification. Cambridge University Press, 1990; 2nd edn.
10. Cutler JE. Putative virulence factors of *Candida albicans*. Annu Rev Microbiol 1991; 45: 187–218.
11. Awad FI, El Molla A, Fayed A, Abd el-Halim M, Refai M. Studies of mycotic mastitis in Egypt. J Egypt Vet Med Ass 1980; 40(3): 35–41.
12. Yeo G, Choi WP. Studies on yeast-like fungi associated with bovine mastitis, 1: Epidemiological study; 2: Sensitivity of yeast-like fungi to antifungal agents. Korean J Vet Res 1982; 22: 121–124.
13. Aalbaek B, Stenderup J, Jensen HE, Valbak J, Nylin B, Huda A. Mycotic and algal bovine mastitis in Denmark. APMIS 1994; 102: 451–456.
14. Krukowski H, Tietze M, Majewski, Rózanski P. Survey of yeast mastitis in dairy herds of small-type in the Lublin region, Poland. Mycopathologia 2000; 150: 5–7.
15. Farnsworth RJ, Sorensen DK. Prevalence and species distribution of yeast in mammary glands of dairy cows in Minnesota. Can J Comp Med 1972; 36: 329–332.
16. Chengappa MM, Maddux RL, Greer SC, Pincus DH, Geist LL. Isolation and identification of yeasts and yeastlike organisms from clinical veterinary sources. J Clin Microbiol 1984; 19: 427–428.
17. Lagneau PE, Lebtahi K, Swinne D. Isolation of yeast from bovine milk in Belgium. Mycopathologia 1996; 135: 99–102.
18. Galli G. Observations and studies on bovine mycotic mastitis. Veterinaria Italiana 1954; 5: 587–604, apud Lohan CB, Kalra DS, Chauran US. Pathology and pathogenesis of experimental mycotic mastitis, I: *Cryptococcus neoformans* infection. Aryana Agricult Univ J Res 1977; 7: 67–72.

Address for correspondence: José Moacir Marin, Departamento de Morfologia, Estomatologia e Fisiologia/FORP/USP, Avenida do Café s/n^0 Campus USP, Ribeirão Preto, São Paulo, Brazil, 14040-904
Phone: 55 16 6024035; Fax: 55 16 633 0999
E-mail: jmmarin@rge.fmrp.usp.br