

PREVALENCE OF MYCOTIC MASTITIS IN COWS

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(Received 15. January 2002)

The aim of this study, performed between 1997 and 2000, was to evaluate the prevalence of bovine yeast udder infections and the accuracy of laboratory diagnostic methods for the isolation and determination of different genera of yeast in milk.

*The results from culture of milk samples from 9754 functional udder quarters are presented. Samples were taken from clinically and subclinically infected udder quarters of 2483 cows. The samples were cultured on blood and Sabouraud dextrose agar (SDA). The final diagnosis was made using the results of the API 20 C and API 20 CAUX (Bio Merieux) systems. Besides bacterial udder pathogens, 187 yeast and 34 prototheca strains were isolated from infected bovine mammary glands. The most prevalent species were: *Candida krusei*, *C. rugosa*, *C. glabrata* and *C. albicans*.*

Key words: algae, cows, mastitis, yeasts

INTRODUCTION

Mycotic mastitis also existed in cattle before the arrival of antibiotics. However, since then there has been an ever increasing number of cases reported, almost always associated with prior antibiotic treatment of suspected or proven bacterial mastitis (Lagneau *et al.* 1996).

Isolation of yeastlike fungi from bovine milk was first reported in 1901. Many earlier reports of yeast mastitis did not include species identification, but as more attempts at identification were made, numerous species were isolated.

The frequency with which potentially pathogenic yeast can be isolated from routine milk samples has been shown to be about 7% in central and northern Europe and also in the USA. In tropical countries, the percentage can be higher (Aalbaek *et al.* 1994).

Mycotic infections of the mammary gland usually occur as sporadic cases affecting a small percentage of cows, or as outbreaks affecting the majority of animals. In both situations, however, the seriousness of infection depends on the number of organisms present in the glands and the species of yeast involved (Farnsworth, 1977).

Yeasts are a group of unicellular organisms, ever present in the natural surroundings of dairy cattle and are normal inhabitants of the skin of the udder and teats, in which they exist in low numbers. They can invade mammary glands,

where they are opportunistic, producing disease when normal defense mechanisms are lowered or when they gain entrance in large numbers. Sources of outbreaks include contaminated antibiotic preparations and inadequate preparation of teat ends prior to treatment. Silage may be the source of lactate-assimilating yeasts known to cause mastitis, especially *Candida krusei*.

Treatment of mycotic mastitis is rarely successful (Bertschinger, 1964, . Elad *et al.* 1995).

MATERIAL AND METHODS

Sampling: A total of 9754 milk samples collected from udder quarters of 2483 cows were examined. The samples were part of our routine mastitis diagnostics procedure and were obtained from clinically and subclinically affected quarters.

The milk samples were always taken aseptically, kept at a temperature of 4°C and plated, at the latest, 24h after sampling.

Bacteriological examination: For bacteriologic examination of milk from the affected cows, samples were inoculated on to blood agar and incubated at 37°C for 48 hours. Bacteria were identified by standard methods using commercial systems. When substantial growth of fungi was noticed, yeast colonies were inoculated on to Sabouraud dextrose agar (SDA) plates, which then were incubated at 30°C for 48 hours. Isolates were subsequently identified, using the API 32°C system, after inoculation into Sabouraud dextrose broth and incubation at 25°C for 24 hours. In the case of *Candida* species, subcultures were inoculated on to selective and differential media (BBL CHROMagar Candida, Becton Dickinson).

RESULTS

The number of udder infections, where yeast or algae were identified has increased over the last few years (Table 1).

Table 1: Number of cases of mastitis where yeasts or algae were isolated

Year	1991	92	93	94	95	96	97	98	99
Number of samples	2500	3167	1412	1615	1958	3069	3334	2875	3545
Number of isolates	5	19	24	21	47	89	149	156	244
% of mastitis due to yeast or algae	0.2	0.6	1.7	1.3	2.4	2.9	4.5	5.4	6.9

The three-year examination (1997 - 1999) of mammary secretions from Slovenian cattle with clinical or subclinical mastitis revealed 549 strains of yeast

and algae. The strains originated from 527 mammary secretions of 483 cows in 127 herds. A total of 221 strains was identified at the species level. The species of yeasts and algae identified are shown in Table 2.

Table 2: Frequency of individual genera of yeasts and algae, isolated from mammary secretions

Genus/ Species	No of isolates	%	
<i>Candida krusei</i>	75	34	
<i>Candida rugosa</i>	25	11	
<i>Candida tropicalis</i>	22	10	
<i>Candida albicans</i>	19	9	
<i>Candida kefyr</i>	18	8	% yeasts - 84.6
<i>Candida guilliermondii</i>	13	6	
<i>Candida glabrata</i>	7	3	
<i>Candida parapsilosis</i>	4	2	
<i>Cryptococcus neoform.</i>	4	2	
<i>Prototheca zopfii</i>	34	15	% algae - 15.4
Total	221	100	

DISCUSSION

The isolation of yeasts and algae from 1997 to 1999 inclusive at an average prevalence of 5.6%, in secretions of cows with clinical or subclinical mastitis is comparable with the results obtained in a previous Slovenian survey (4.1%) (Pengov and Zdovc, 1998).

The predominant eukaryotes identified in this study were *Candida* species, making up 183 of 221 strains. This is in accordance with results reported from other countries (Lagneau *et al.* 1996).

The species *C. krusei* accounted for more than one third of the strains isolated. *C. krusei* was also the predominant species in some comparable studies (Pengov and Zdovc, 1998, Topolko, 1968), whereas *C. tropicalis* was the most important in others (Richard *et al.* 1980). In several surveys of mycotic mastitis *C. tropicalis*, *C. rugosa*, and *C. krusei* were often demonstrated (Farnsworth and Sorensen, 1972).

The algal genus *Prototheca* includes unicellular immotile asexual algae without chlorophyll. The colony morphology of *Prototheca* species is indistinguishable from that of yeasts. *Prototheca*-associated bovine mastitis was first described by Lerche and since then the organism has been isolated a number of times. The epidemiology of bovine *Prototheca*-associated mastitis may be endemic or sporadic (Frank *et al.* 1969). The 34 *Prototheca* strains noted in the present study originated from cows in separate herds.

The finding of microorganisms in a mammary secretion may reflect a population established in the gland, one that is harboured in the streak canal (ductus papillaris), or simply external contamination. Mammary infection is

defined by the demonstration of microorganisms together with an inflammatory reaction.

The yeasts and algae from the present material fulfill this condition. Their significance is further supported by the marked inflammatory reactions of most secretions as well as the absence of other microorganisms in most secretions.

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RAŠIRENOST GLJIVIČNOG MASTITISA KOD KRAVA

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SADRŽAJ

Svrha ovih ispitivanja izvršenih između 1997 i 2000 godine je bila da se oceni proširenost gljivične upale vimena kod krava i pouzdanost laboratorijskih dijagnostičkih metoda za izolaciju i determinaciju različitih vrsta gljiva u mleku. Analiza je izvršena na osnovu rezultata bakterioloških analiza uzoraka mleka iz 9754 četvrti vimena krava. Uzorci su bili uzimani u slučajevima kliničkih i subkliničkih upala vimena i nanošeni na krvni i Saubouraud dekstrozni agar (SDA). Završna diagnostika je izvana sa API 20 C i API 20 C AUX (Bio Merieux) sistemom. Pored bakterijskih uzročnika upale vimena, iz zaraženih četvrti smo izolovali još 187 sojeva gljivica i 34 sojeva algi. Najčešće su bile izolovane sledeće vrste: *Candida krusei*, *C. rugosa*, *C. glabrata* i *C. albicans*.