Prevalence and Species Distribution of Yeast in Mammary Glands of Dairy Cows in Minnesota

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SUMMARY

A study of the prevalence of yeast-like fungi in the mammary glands of dairy cattle was conducted in Minnesota. Quarter samples from 6,020 cows were cultured for yeast. Growth of organisms was obtained from 3.2% of the quarter milk samples. The rate of yeast infection for Minnesota dairy cattle in this study was 2.0%.

The majority of the yeast isolated belonged to one of four species of the Candida genus. Candida krusei, Candida parakrusei, Candida guilliermundi, and Candida tropicalis, comprised 89% of the yeasts isolated. All of these species have been reported to cause clinical mastitis (1, 7, 9, 10, 12, 13, 15, 16).

It would appear that yeast-like fungi are of sufficient prevalence in mammary glands that yeast infection would be considered in the differential diagnosis in cases of clinical mastitis.

RÉSUMÉ

Les auteurs ont effectué, au Minnesota, une étude sur la prédominance de champignons voisins des levures, dans le pis de vaches laitières. Ils recherchèrent des levures, au moyen de cultures, dans des échantillons de lait provenant de quartiers de 6,020 vaches. Ces échantillons donnèrent des résultats positifs, dans une proportion de 3.2%. Au cours de cette étude, le taux d'infections à levures chez les vaches laitières du Minnesota s'établissait à 2.0%.

La majorité des levures isolées appartenaient à quatre espèces du genre Candida. En effet, Candida krusei, Candida parakrusei, Candida guilliermundi et Candida tropicalis représentaient 89% des levures isolées. On a déjà rapporté que toutes ces espèces peuvent causer une mammite clinique (1, 7, 9, 10, 12, 13, 15, 16).

Il semble que les champignons voisins des levures sont si prédominants dans les glandes mammaires qu'il faut penser à une infection à levures dans le diagnostic différentiel des cas de mammite clinique.

INTRODUCTION

The eradication of *Streptococcus agalactiae* is considered an important procedure in the control of bovine mastitis and necessary for a profitable milk production enterprise (5, 9, 17). One of the problems sometimes encountered in *S. agalactiae* eradication programs is infection with yeast-like fungi (5, 6). These infections, while not a problem in every herd, happen with sufficient frequency to be of concern. Clinical mastitis due to yeasts may occur in connection with the treatment phase of a *S. agalactiae* program. Yeast mastitis epizootics have been traced to the use of yeast contaminated antibiotic preparations (1, 12). Other sources of yeast infection exist since some infections have occurred in cows with a history of no intramammary use of antibiotics (4, 10, 17).

Cases of clinical mastitis due to yeast have occurred in the herds participating in

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the mastitis control programs conducted by the Department of Veterinary Medicine at the University of Minnesota (5). In view of these problems a study was initiated to determine the extent of subclinical yeast infection in the mammary glands in dairy animals in Minnesota.

MATERIALS AND METHODS

Source of milk samples for this study was the mastitis research laboratory maintained by the College of Veterinary Medicine at the University of Minnesota. A large portion of the routine samples processed by this laboratory were obtained from herds involved in the mastitis programs being conducted as part of a research project. Samples from clinical cases of mastitis encountered by staff members of the Ambulatory Clinic, the Large Animal Clinic, and some practicing veterinarians were also processed by this laboratory. Quarter samples from 6,020 cows were examined. Samples from 81 cows were submitted by the staff of the Ambulatory Clinic and practicing veterinarians submitted samples from 643 cows. The samples from the remaining 5,396 cows were obtained from the herds involved in the mastitis control program.

The milk samples were obtained as aseptically as possible under field conditions. The cow's udder was brushed or washed and dried and the teat ends were cleaned with cotton pledgets saturated with 70% isopropyl alcohol. The first stream of milk or approximately 5 cc was discarded and the sample was then collected. Quarter samples of approximately 12 to 15 cc were obtained in screw cap culture tubes 16x125 mm. Since the same samples were used for the Hotis test, the tubes contained 0.5 cc of a 0.5% solution of brom cresol purple. The milk samples were incubated at 37°C in the culture tubes for approximately 18 hours. Following incubation the samples were mixed and subjected to bacteriological examination. At this same time a calibrated loop was used to streak .01 cc over one-fourth of a 100 mm plate containing Sabouraud's dextrose agar containing 300,000 units of aqueous penicillin and 500 mg dihydrostreptomycin in each 500 cc of agar. The Sabouraud's plates were incubated at 37°C for 48 hours and a sample of any growth obtained was collected for yeast identification. The incubation temperature of 37°C was used because it has been observed that strains of yeast isolated from mastitis cases grew more abundantly at this temperature than at lower temperatures, while nonpathogenic yeast did not usually grow at 37°C (14).

The methods followed and the types of media used were similar to recommendations found in the *Laboratory Manual for Medical Mycology* (17). To identify the species of yeast isolated, the growth obtained on the Sabouraud's dextrose agar was inoculated on two different types of media and sugar fermentation tests were conducted. Tryptose blood agar plates containing 5% bovine blood were inoculated and incubated for 24 hours at 37°C. Gram stains were made of the growth obtained to determine if any bacterial contamination was present. Pagano's Medium[1] was inoculated and incubated at 25°C. Observations were made noting color and colony type as growth occurred. The interpretations of color reactions on Pagano's Medium are listed as follows:

Reactions on Pagano's Medium

Genus *Candida*	Color Reaction
C. albicans	Cream to light red
C. krusei	White (spreading)
C. parakrusei	Light pink
C. tropicalis	Deep red
C. pseudotropicalis	Pink
C. guilliermundi	Light red
C. stellatoidea	Red

Samples of the isolates were inoculated into tubes containing Tryptose broth and a 1% concentration of dextrose, lactose, sucrose, or maltose. These tubes were equipped with small vials for gas collection and contained the pH indicator phenol red. After inoculation, one-fourth inch of sterile mineral oil was layered on the top of the sugar solution in each tube to prevent air contact. The fermentation tubes were incubated at 37°C for ten days. Observations were made on days 1, 2 and 3 after inoculation and every three days thereafter. A tube of Sabouraud's dextrose broth was also inoculated and observations were made for surface growth. These tubes were also incubated at 37°C. Tubes of sterile bovine serum were inoculated with each isolate and incubated at 37°C. Observations were made for chla-

[1]Difco Laboratories, Detroit, Michigan.

mydosphores after five hours by the use of direct microscopic examination of specimens stained with lactophenol cotton blue. Originally, corn meal agar was used but later in the study the method using bovine serum was employed (2).

Any isolates which were not identified by the above process were inoculated into Christensen's urea media and observations made for a deep red color at 48 hours. If the isolates were negative for urease production no further identification was attempted. If urease was produced the isolate was considered to be a suspect for *Cryptococcus* spp. and was submitted to the State of Minnesota Diagnostic Laboratory for pathogenicity tests and further identification. Some isolations were made which showed the morphology of true fungus but further identification was not attempted.

RESULTS

During a two-year period 23,960 quarter milk samples from 6,020 cows were surveyed for yeast-like fungi by inoculating Sabouraud's agar containing penicillin and dihydrostreptomycin. Growth occurred on the plates from 783 quarters of 588 cows. These data are shown in tabular form in Table I.

Identification of the species of yeast-like fungi was attempted on only approximately 40% or 372 of the Sabouraud's isolates. Identification of the isolates from 13 quarters was not accomplished because the yeast failed to grow on subculture or became contaminated with various fungi which interfered with further tests. Samples from another 13 quarters yielded antibiotic resistant bacteria. Nineteen Sabouraud isolates were found to be true fungi. The remaining 327 of these 372 isolates were identified as yeast but we were unable to determine the species of 20 of this group. The results of the species' identification are presented in Table II.

TABLE I. Results of Study to Determine the Incidence of Yeast-like Fungi in Milk Samples of Minnesota Dairy Cattle[a]

Item	Number Sampled	Number Positive	Percent Positive
Cows	6,020	588	9.7
Quarters	23,960	783	3.3

[a]Based on Sabouraud's Agar Cultural Procedures

TABLE II. Distribution of Identified Yeasts in a Selected Group of Samples

	No. of Cows Infected	No. of Quarters Infected	% of Total Yeast
Candida krusei	132	166	50.8
Candida parakrusei	55	63	19.3
Candida guilliermundi	33	41	12.5
Candida tropicalis	17	22	6.7
Candida pseudotropicalis	7	8	2.5
Candida albicans	5	5	1.5
Candida stellatoidea	1	1	0.3
Cryptococcus spp.	1	1	0.3
Unidentified	19	20	6.1
	270	327	100.0

DISCUSSION

The pathogenesis of yeast mastitis has not been well determined. Clinical mastitis has been reported on numerous occasions but the source of infection usually has not been identified. In some instances yeast contaminated intramammary treatment preparations have been incriminated but in other cases such preparations were not involved. This study was conducted to determine the number of times that yeast may be recovered from clinically normal animals, It was shown that pathogenic species of yeast could be found in the mammary gland or teat canal of 2.02% of the quarters examined. This finding would indicate that there are additional factors involved in the development of clinical yeast mastitis since clinical infections are not extremely common.

In some samples, an actual infection may not have been present within the gland. The possibility of a yeast contaminant growing during the incubation period exists. The presence of increased leukocyte count is often used as a criteria for infection or pathogenicity, but in previously conducted, unpublished herds' studies, it was observed that yeast could be repeatedly isolated from the same quarters of the same cows with no increase in leukocyte count. It was also noted in these herds that the isolation of yeast followed a very consistent pattern when repeated samples were taken. It would be expected that if isolation of yeast

were due to contamination the pattern would be quite random. Since the method used in these unpublished studies were similar to those discussed in this paper, some evidence is presented to support the theory that many of the yeasts isolated were within the mammary gland or teat canal. Those yeasts isolated also grew at 37°C which presents some evidence for pathogenicity (8). Further evidence for pathogenicity is present for some strains of yeast in this study since clinical yeast mastitis was observed in some of the herds and clinical yeast mastitis was produced experimentally using strains obtained from field cases.

Although there are numerous reports of *Cryptococcus* spp. causing clinical mastitis, only one isolate of this genus was made during this study. *Cryptococcus* spp. have been reported as causing a rather severe clinical mastitis and since this study was concerned primarily with subclinical infection, fewer *Cryptococcus* isolates were expected. The cultural methods used may also have been more effective in isolation of the *Candida* spp.

It is apparent from the results of this study that yeast-like fungi are present in the mammary gland and on the teat surfaces in sufficient numbers of cows to allow the development of yeast mastitis if the other factors necessary for infection are present.

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