NOTES

Isolation and Identification of Yeasts and Yeastlike Organisms from Clinical Veterinary Sources

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A total of 229 isolates of yeasts and yeastlike organisms recovered from a variety of clinical specimens were identified by using the API 20C microsystem in conjunction with morphological characteristics and urea hydrolysis. Of the 229, 218 (95.1%) were from bovine, porcine, canine, and equine species and the remaining 11 (4.9%) were from feline and avian species. The gastrointestinal and reproductive tracts were the major sources of yeasts and yeastlike organisms, representing 60 (26.2%) and 28 (12.2%) isolates, respectively.

Yeasts are ubiquitous in nature and are frequently isolated from the gastrointestinal tract of humans and other animals. Only a small number of species have been implicated as causal agents of disease in animals (1–3, 7, 9, 11, 12). In contrast, a wide variety of yeasts and yeastlike organisms have been involved in infections of humans (6). One contributing factor is the intensive and prolonged use of antibiotics which disrupt the microflora that serve as one of the natural defense mechanisms of the host (1, 9).

The repeated recovery of a potentially pathogenic yeast in nearly pure culture or in large numbers provides strong evidence of its pathological significance. Yeasts and yeastlike fungi have been associated with clinical mastitis in dairy cattle (3). A majority of the yeast isolates from bovine clinical mastitis belong to the genus Candida (3, 9).

Yeasts and yeastlike fungi have also been implicated as causes of bovine reproductive problems, including abortion in cows and infertility in bulls (8, 11, 14). The results of recent studies have indicated the involvement of yeastlike fungi as primary pathogens in canine ear and urinary tract infections (2, 7, 12) and in equine uterine infections (1). The ulcerogenic potential of Candida spp. in foals has been reported recently by Gross and Mayhew (5). Their findings suggested that species of Candida might have predisposed foals to gastric ulcerations by weakening the squamous mucosa through loss of the outer protective keratin layer.

The purpose of this study was to isolate and identify yeasts and yeastlike organisms from various clinical specimens submitted to the Breathitt Veterinary Center for a period of 26 months. The alga Prototheca zopfi was also included in this study because of its close similarity to yeasts in colony characteristics and growth requirements for primary isolation of yeasts.

A total of 229 isolates were used in this study. These isolates were recovered from clinical material of veterinary origin at the Murray State University Breathitt Veterinary Center. Specimens were collected from a variety of sources in the six animal species shown in Table 1.

Specimens were cultured on Sabouraud glucose agar or tryptic soy blood agar (Difco Laboratories, Detroit, Mich.) and eosin-methylene blue agar plates (BBL Microbiology Systems, Cockeysville, Md.) The plates were incubated at 37°C (Sabouraud glucose agar at 25°C) and examined at 24 and 48 h for the presence of yeasts and yeastlike organisms. When recovered, these isolates were streaked onto Sabouraud glucose agar plates and incubated at 30°C for 72 h. These 72-h cultures were used as sources of inocula for the identification procedure.

Isolates were identified by using the API 20C microsystem (API 20C, Analytab Products, Plainview, N.Y.) in conjunction with morphology and urea hydrolysis. In the event that a definitive identification could not be reached by methods listed above, additional tests were set up which included KNO₃ assimilation, carbohydrate fermentations, ascospore induction, and growth on Sabouraud glucose agar at 42°C for 72 h (13). Those isolates that could not be definitely identified by the criteria listed above were sent to the Mycology Reference Laboratory at Analytab Products for further testing.

Table 1 shows the number of yeasts and yeastlike organisms recovered from various clinical specimens of six different animal species. A great majority (95.1%) of the isolates recovered in this survey were from bovine, porcine, canine, and equine species. The gastrointestinal and reproductive tracts of these animals were the major source of yeasts and yeastlike fungi (26.2 and 12.2%, respectively), whereas bovine mastitic milk samples were the only source of P. zopfi (Table 1).

The pathogenesis of yeasts and yeastlike fungi has not been well documented in the animal species. A great majority of them are considered saprophytes; however, in a few instances they are considered potential pathogens. The repeated isolation of yeasts or yeastlike fungi is often used as a criterion for infection or pathogenicity.

Although the pathogenicity of P. zopfi has not been studied thoroughly, our results indicate that the mastitis in 10 cases was probably caused by P. zopfi. The cultural examination of these milk samples did not reveal the involvement of bacteria as the cause of mastitis. Chemotherapeutic agents used in the treatment of these mastitic cases

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were found to have no curative effect. This further supports the hypothesis that *P. zopfii* was probably the causal agent of clinical mastitis. Similar findings have been reported by Frank et al, who found that *P. zopfii* was the sole isolate in mastitic milk samples (4).

This survey reveals that *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* were the most frequent species recovered from clinical specimens representing 71.4% of total *Candida* isolates. The less frequent species of *Candida* recovered represent 28.6% of the total *Candida* isolates and included the following: *C. blankii*, *C. ciferrii*, *C. freysschussii*, *C. guilliermondii*, *C. lusitaniae*, *C. pelliculosa*, *C. pseudotropicalis*, *C. ravautii*, *C. rugosa*, *C. utilis*, and *C. zeylanoides.* The remaining important yeasts and yeastlike isolates identified in this study included species of *Cryptococcus, Hansenula, Malassezia, Prototheca, Rhodotorula, Torulopsis,* and *Trichosporon.* In the present study, attempts were not made to correlate the presence of yeasts to disease conditions. However, several case reports in recent years have indicated yeasts as primary pathogens in domestic animals (1–3, 7–9, 11, 12). Even as commensals, yeasts and yeastlike fungi are extremely important from an epidemiological point of view since a shift from the role of harmless commensal to that of virulent pathogen may be induced by any number of factors.

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### LITERATURE CITED


