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Authors: Irokanulo, E. O. A., Makinde, A. A., Akuesgi, C. O., and Ekwonu, M.

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Cryptococcus neoformans var *neoformans* Isolated from Droppings of Captive Birds in Nigeria

E. O. A. Irokanulo,[1] A. A. Makinde,[1] C. O. Akuesgi,[2] and M. Ekwonu,[3] 1 National Veterinary Research Institute, Vom, Plateau State, Nigeria; **2** Botany Department, Faculty of Natural Sciences, University of Jos, Jos; **3** Jos Zoological Garden, Jos, Plateau State, Nigeria

ABSTRACT: The yeast, *Cryptococcus neoformans*, was found in apparently healthy birds at the Jos Wildlife Park and Zoo in Jos, Nigeria. *Cryptococcus neoformans* var *neoformans* was isolated from feces of four captive bird species. Five isolates belonged to serotype A while two were serotype D. Serotype A of *C. neoformans* was isolated from a white face duck (*Dendrocygna viduata*), eagle owl (*Bubo africanus cinerascene*) and peacock (*Pavo cristatus*). The other two (serotype D), were isolated from a spotted eagle owl.

Key words: *Cryptococcus neoformans*, captive birds, Wildlife Park, Zoo.

The encapsulated yeast, *Cryptococcus neoformans*, is widespread in nature (Jawetz et al., 1982). The four known serotypes of this species have been divided into two groups based on serologic groupings and epidemiological differences: *C. neoformans* var *neoformans* (serotype A and D) and *C. neoformans* var *gatti* (serotypes B and C) (Kwong-Chung and Bennett, 1984; Wilson et al., 1986). All serotypes are found in most parts of the world and all are most commonly recovered from clinical materials. Serotypes A and D, unlike the B and C serotypes, are commonly associated with bird droppings and soil where potential birds carriers such as the pigeon, nest (Bennett et al., 1977). The natural source of the *C. neoformans* var *gattii* variety has remained unknown until recently when an association between the var *gatti* serotypes and the soil as well as vegetation around the *Eucalyptus camaldulensis* trees was highlighted (Ellis and Pfeiffer, 1990).

Reports on bird species that harbor *C. neoformans* in their feces have been varied, with the pigeon (*Columba* species) remaining the principal carrier (Emmons, 1955; Ajello et al., 1967; DiSalvo, 1987). Our objective was to evaluate the birds at the Jos Zoo and Wildlife Park, Nigeria, for the presence of *C. neoformans* var *neoformans*.

The feces of 15 bird species kept at the Wildlife Park and Zoo in Jos, Nigeria, were collected and cultured: one each in the dry season month of February, March, April, November, and December 1991, and January 1992 (Table 1). Dry and freshly collected specimens were suspended in 5 ml of sterile phosphate buffered saline (PBS) and left to stand for 3 hr to dissolve the hard and caked droppings. These were shaken vigorously on a mechanical shaker and filtered through a sterile muslin gauze. The filterate was centrifuged at 2,000 × *G* for 5 min. The pellets formed were used to inoculate duplicate plates of Sabourand dextrose agar (SDA) (Biotec Laboratories, Lightwater, Surrey, United Kingdom) containing 0.05 mg/ml chloramphenicol and on the Niger seed (William G. Scarlet and Company, Baltimore, Maryland, USA) agar plates. The seeded plates were incubated at 25C and 37C for 6 days. Small cream or off-white colonies which grew on SDA and brown-tan colonies on the Niger seed agar media were stained by the Grams method and large Gram-positive coccoid cells were considered as yeast. A wet India ink preparation of the suspected colonies earlier subcultured unto fresh SDA and incubated for 72 hr was carried out and examined for presence of capsules and the yeast-like cells which showed presence of capsules were further tested for their sugar fermentation and assimilation patterns (Baker and Breach, 1980) and also for their ability to hydrolyze urea (Cowan, 1975). The encapsulated yeasts were confirmed as *C. neoformans* based on their ability to assimilate the following car-

TABLE 1. Bird species investigated for *C. neoformans* between January 1991 and 1992 in Jos, Nigeria.

Bird species	Source	
	Wildlife	Zoo
White-face duck (*Dendrocygna viduata*)	0	2
Tawny eagle (*Aquila rapax*)	0	2
African river eagle (*Cancuma vocifer*)	2	5
Red-tailed buzzard (*Buteo auguralis salvadori*)	0	2
Bateleur eagle (*Tertergthopius ecaudatus*)	0	6
West African black kite (*Milvus migrans*)	2	4
Spotted eagle owl (*Bubo africanus cinerascene*)	0	8
Marabou stork (*Leptoptilos crumeniferus*)	0	1
Grey parrot (*Psittacus erithacus erithacus*)	0	2
Peacock (*Pavo cristatus*)	1	0
Peahen (*Pelecanus rufescens*)	2	0
American white pelican (*Pelecanus erythrohynchos*)	1	0
Milky eagle owl (*Bubo lacteus*)	1	3
Spur-winged goose (*Plectropterus gambensis gambensis*)	0	2
White-backed vulture (*Gyps bengalensis*)	6	0

bohydrates: dextrose, maltose, sucrose, galactose, cellobiose, inositol, xylose, raffinose, trehalose and dulcitol, but not lactose or melibiose, as well as their inability to reduce nitrate or ferment dextrose, maltose, sucrose, lactose, galactose and trehalose (Love et al., 1985). On primary isolation, all the *C. neoformans* possessed moderate capsules.

Serotype differentiation of the *C. neoformans* yeasts was performed using the creatinine dextrose bromothymol blue (CDB) agar (Kwong-Chung et al., 1978); and canavanine glycine bromothymol blue (CGB) agar (Kwong-Chung et al., 1982). Based on serotype differentiation using the CDB and CGB, all isolates belonged to the *C. neoformans* var *neoformans* group. A further differentiation of the var *neoformans* group into serotypes A and D was carried out using creatinine dextrose bromothymol blue thymine medium (Irokanulo et al., 1994).

Seven (13%) of 52 captive birds belonging to 15 species that had their droppings cultured were infected with *C. neoformans*. Five isolates belonged to the var *neoformans* serotype A and two were serotype D. Six of the isolates were from the bird droppings collected from the Jos Zoo: one each from the white face duck (*Dendrocygna viduata*) and the African river eagle (*Cancuma vocifer clamos*), and four from a spotted eagle owl (*Bubo africanus cinerascene*). The only isolate made from the wildlife park came from the droppings of a peacock (*Pavo cristatus*). None of the birds whose excreta were examined had clinical symptoms at the time of this study. There was no evidence that any of the birds had suffered from any mycotic infection for the 6 mo prior to this investigation. On physical examination of the birds we did not find such symptoms as fluffed feathers, closed eyes, reduced activity, drooping wings, abnormal breathing patterns, dry flaky skin, feather lose, changes in droppings and weight loss. The isolation of *C. neoformans* from the droppings of the captive birds therefore is evidence that these birds may be potential carriers for the var *neoformans* serotypes. The recovery of *C. neoformans* var *neoformans* serotype only, particularly the serotype A from the droppings examined further is evidence that the natural site for the *C. neoformans* var *gattii* serotype may not be completely related to birds. Though the specimens were collected from each bird's cage at six different periods of the year, the prevalence of the *C. neoformans* var *neoformans* remained relatively low (13%).

Two reasons may explain this finding: the good management and care for the birds and the fact that the droppings and debris in the pens were not allowed to accumulate. Isolations were made only in the months of December, January, and February. These three months constitute the coldest months of the year in Jos. It therefore becomes necessary to conduct more elaborate studies on the relationship between the *C. neoformans* yeast's existence and environmental temperature in Nigeria considering an earlier report on the possible existence of definite soil temperature range for the genus *Cryptococcus*; this relationship may differ from one region or coast to another (Sneller and Swatek, 1974). The observations made from this investigation have again highlighted the presence of the encapsulated pathogenic yeast in birds that are not commonly reported to be associated with the haboring of *C. neoformans*.

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