Case Report

First case of cryptococcosis in a new species of bandicoot (*Bandicota indica*) caused by *Cryptococcus neoformans* var. *grubii*

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The first case of cryptococcosis caused by *Cryptococcus neoformans* var. *grubii* in a new species of bandicoot (*Bandicota indica*) is described. The animal was trapped in a bamboo thicket in a park located in the city of Jabalpur, India. On necropsy, pathological lesions were seen in the lungs and liver and *C. neoformans* var. *grubii* was isolated from the lungs, liver, kidneys, spleen and brain but not the heart or intestine. The soil of the animal’s burrow and bamboo debris around it also revealed the presence of *C. neoformans* var. *grubii*. We hypothesize that the bandicoots may potentially act as sentinel animals for environmental human pathogenic *Cryptococcus* species.

**Keywords** *Bandicota indica*, natural host, *Cryptococcus neoformans* var. *grubii* (serotype A)

Introduction

*Cryptococcus neoformans* is an exogenous, opportunistic pathogen capable of causing life-threatening infections, especially in persons with cellular immunodeficiencies, such as those with AIDS, organ transplants, or hematologic malignancies [1]. On the basis of differences in ecology, physiology, capsular polysaccharide and clinical manifestation, two species, one with two varieties and five serotypes of pathogenic *Cryptococcus* have been recognized. The most common, *Cryptococcus neoformans* var. *grubii* represents isolates of serotype A. It primarily infects immunosuppressed individuals and causes more than 90% of all cryptococcal infection and more than 99% of the cases in patients with AIDS [1,2]. Isolates of two serotypes, B and C of *Cryptococcus gattii* tend to infect immuno-competent individuals. The yeast is ubiquitous in the environment, where it is usually associated with avian guano or plant debris [3–5]. The species is usually confined to tropical regions where it is associated with decaying *Eucalyptus* and other trees [1]. Strains of serotypes A and D are commonly isolated from pigeon excreta [3,6,7] or soil contaminated with weathered bird excrement [8]. It has been suggested that this is because avian excreta has a high concentration of low molecular weight nitrogen compounds which aid in the growth of the pathogen [9]. In addition, several reports describe the isolation of strains of *C. neoformans* var. *grubii* from decaying trees [4,7] and household dust [1,7,10]. Randhawa et al. [4] also added to the recently emerging evidence that the natural habitats of *C. gattii* and *C. neoformans* are not specific to woody or other debris of particular tree species but instead are more generalized. Kidd et al. [11] isolated *C. gattii* on Vancouver Island (Canada) from many species of trees and vegetative debris but not from eucalyptus trees and suggested that the ecological niche of the pathogen is much broader than previously thought.
Recently, Duncan et al. [12] reported that the relative proportion of nasal colonization of *C. gattii* in wild animal species is consistent with findings in domestic animals and suggested that animals may be a good indicator of environmental organisms. In the present study, we report a case of cryptococcosis in a new species of bandicoot, *Bandicota indica* caused by *C. neoformans* var. *grubii* and its ecological and epidemiological significance.

**Materials and methods**

As part of an investigation related to extra human sources of human pathogenic fungi in the environment of Jabalpur, a town of Central India, two Bandicoot rats (*Bandicota indica*) were captured from their burrows in a bamboo thicket located in a local park (Fig. 1). After clinical and behavioral examinations of the animals, they were euthanized, their organs aseptically removed and pathological lesions, if any, noted. Subsequently, lungs, liver, kidneys, spleen, brain were cut into two equal halves. One half of the samples from each organ were fixed in 10% formalin and the other half homogenized in sterile normal saline. A drop of the tissue homogenate of each organ was placed on the glass slide in 10% KOH stained with cotton blue and India ink and observed under the microscope for the presence of any capsulated yeast cells. Simultaneously, 0.5 ml of the homogenate was spread on the surface of triplicate plates containing Sabouraud’s dextrose agar (SDA) with 0.05 mg/ml Chloroamphenicol and incubated at 28±1°C for a week. The cultures were observed daily and colonies counted with a colony counter to determine the colony forming units per ml. For histopathology studies, tissue samples fixed in formalin were dehydrated, embedded in paraffin wax, 5 μm sections were cut and stained with Gomori’s Methenamine Silver Nitrate (GMS) and Hematoxylin and Eosin (HE).

Two samples, each consisting of 10 g of burrow soil and bamboo debris, were aseptically collected in February and April 2001 and stored at 10°C. Within 48 h of collection, 1g each of these samples were suspended in 10 ml of sterilized distilled water, shaken vigorously for few minutes and allowed to stand for 20 min. From each of the latter, 0.1 ml of supernatant were separately streaked on to triplicate plates of SDA and Staib’s agar media with chloroamphenicol (0.05 mg/ml) and incubated at 37°C. Plates were examined daily and colonies counted to determine cfu/ ml. Mean cfu/ ml was calculated and SD determined. The isolates from the organs of the animal, burrow soil and bamboo debris were subcultured onto Staib’s agar medium supplemented with chloroamphenicol (0.05 mg/ml), 0.1 g of biphenyl (0.1 g/10 ml of 95% ethanol) per liter and incubated at 30°C for 5 days. Brown yeast colonies were selected, and confirmed as *C. neoformans* using standard morphological and physiological criteria[13]. Isolates were serotyped with commercial monoclonal antibodies (Crypto Check kit Iatron laboratories, Tokyo, Japan).

**Results**

Both animals were robust, healthy males about 35–40 cm in length from nose to base of the tail and approximately 1.4 kg in weight (Fig. 2). On post mortem examination one of the two animals was found to be presumptively positive for cryptococcosis as it exhibited severe pulmonary disease with discrete and confluent, soft, elevated less than 1 mm diameter lesions that were often caseated. All other organs appeared to be normal. Direct microscopy of liver, lungs, kidneys, spleen and brain samples was positive for encapsulated yeastlike cells consistent with those of *C. neoformans*. Heart and intestine were negative for the pathogen. Histopathological section of the lungs revealed diffuse thickening of
interalveolar septa, infiltration of inflammatory cells, destruction of alveolar structure and the presence of foci of histocytes, macrophages and lymphocytes with some incorporating yeastlike cells consistent with those of \textit{C. neoformans} (Fig. 3). Tissue sections of liver showed congested central veins in which the cells were edematous and arranged around it in a habicular pattern. The cytoplasm was granular and nucleus showed prominent nucleoli. The sinusoids were dilated and filled with red blood cells (RBC). At places, focal collection of inflated cells and encapsulated yeastlike cells were seen. The glomeruli in the kidney sections were markedly congested with RBC and hyper cells. There was marked congestion of blood cells in the interstitial space. At one corner intense focal collection of lymphocytes forming non–specific granuloma was visible. Histopathology studies of the brain, spleen, heart and intestine were not performed. In culture, samples of the spleen yielded greatest number of colony forming units (3.5 $\times$ 10$^5$ cfu/g) followed by lungs (3 $\times$ 10$^5$ cfu/g), brain (1.5 $\times$ 10$^5$ cfu/g), kidneys (1 $\times$ 10$^5$ cfu/g), liver 4 $\times$ 10$^4$ cfu/g). All isolates were identified as \textit{C. neoformans} var. \textit{grubii}.

While samples of the animal’s burrow soil and decomposing bamboo material collected on two separate occasions were positive for \textit{C. neoformans} var. \textit{grubii}, there were variations in the yeast population. Samples of soil from inside the burrow collected in February yielded 2.5 $\times$ 10$^6$ cfu/g, while those in April yielded 1.5 $\times$ 10$^6$ cfu/g. Similarly, bamboo plant debris from around the animals’ burrow yielded 2 $\times$ 10$^5$ cfu/g in February and 8 $\times$ 10$^5$ cfu/g in April.

**Discussion**

The genus \textit{Bandicota} contains two species; \textit{B. bengalensis} and \textit{B. indica}. These rodents are commonly referred to as bandicoots and belong to the class

![Fig. 2 Photograph of Bandicota indica.](image)

![Fig. 3 Histopathology of the lung: (a) showing inflammatory reaction and alveolar structure (×60, HE), (b) presence of foci of blood cells incorporating yeast like cells of Cryptococcus neoformans (×100, HE), (c) Cryptococcus neoformans invasion of the lung (×400, GMS stained).](image)
C. gattii, influenza in horses [14]. It is interesting to note [14]. The bandicoots have been associated with the

They build large burrows in the soil and are omnivor-

in or around human dwellings. They are widely
distributed throughout peninsular India from the
Himalayas to Cape Cambrian but are more common
in fields and forest and are associated with man, living
in this region, on the basis of the present finding, it
seems likely that B. indica act as sentinel animals for
environmental human pathogenic Cryptococcus spe-
cies. Nevertheless, additional studies are warranted
which are underway to assess the possibility of
B. indica acting as sentinel species in cryptococcosis
in this region.

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