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ABSTRACT

***Objective*: To establish the environmental reservoirs of *Cryptococcus neoformans* and *Cryptococcus gattii* in Nairobi, Kenya.**
***Design*: Prospective study.**
***Setting*: Kenya Medical Research Institute, Mycology laboratory, Nairobi, Kenya.**
***Subjects*: A total of 400 environmental samples from different sites were analysed including; avian droppings, tree swabs, soil contaminated with avian droppings and swabs from garbage damping sites. Samples were subjected to various phenotypic tests including microscopic morphology, physiological and biochemical tests, pigmentation on bird seed agar and reaction on Canavanine-Glycine-Bromothymol Blue agar.**
***Results*: *Cryptococcus neoformans* was isolated from 23/200 (11.5%) dropping samples and *Cryptococcus gattii* in 5/200 (2.5%) of the same samples. *Cryptococcus gattii* was isolated from 7/60 (11.7%) tree swabs and *Cryptococcus neoformans* in 5/60 (8.5%) of the same samples. From other sites there was no *Cryptococcus gattii* recovered with (5/50: 10%), (6/60: 10%), (2/30: 6.7%) *Cryptococcus neoformans* recovered from chicken cage, garbage damping site and soil respectively.**
***Conclusion*: Findings clearly showed a high presence of *Cryptococcus neoformans* and *Cryptococcus gattii* from several environmental sites in Nairobi, Kenya .This could probably explain the high incidence of cryptococcal meningitis in HIV/AIDS patients in Kenya.**

INTRODUCTION

Cryptococcus neoformans and *Cryptococcus gattii* are pathogenic basidiomycetous yeasts that cause cryptococcosis disease in immunocompromised and immunocompetent patients (1,2). With the emergence of the AIDS pandemic, the incidence of cryptococcosis is increasing and represents a major life threatening fungal infection in these patients (1,3). Globally, the risk of cryptococcal meningitis in HIV / AIDS is estimated between 0.04 to 12% in adults and about 1% in children. The highest population at risk of meningoencephalitis is in sub-Saharan Africa and South East Asia where the highest burden of HIV / AIDS exists (1,4). A low CD4 cell count is the main predictor of risk of cryptococcal meningoencephalitis; the vast majority of cases occur among AIDS patients with a CD4 cell count<100 cells / mm (5).

Cryptococcus neoformans has historically been divided into three varieties of five serotypes based on antigenicity of the capsule: *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *gattii* (serotypes B and C), *C. neoformans* var. *neoformans* (serotype D), and one hybrid (serotype AD) (6). In 2002, C. *neoformans* var. *gattii* (serotypes B and C) was awarded species status and renamed *Cryptococcus gattii* (7). The two varieties exhibit different sexual states; *Fillobasidiella neoformans* var. *neoformans* is the teleomorph of *C. neoformans* var. *neoformans* and *F. neoformans* var. *bacilliospora* is the teleomorph of *C. neoformans* var. *gattii*. The two varieties are morphologically similar except that basidiospores of var. *neoformans* are round and those of var. *gattii* are more elliptical in shape (7). The definitive identification of the two varieties is possible through biochemical tests such as resistance to canavanine and use of glycine as the sole carbon and nitrogen and resistance of their urease enzyme to EDTA (8). *C. neoformans* has a worldwide distribution and has been associated with a variety of environmental sources in particular, bird excreta and decaying wood (9,10). It is documented that

C. gattii is limited to tropics and sub-tropical regions and causes cryptococcosis in over 40 % of non AIDS patients (2,11). Majority of the isolates from Europe belong to C. *neoformans* var. *neoformans* while only 15 % of the isolates from USA, Argentina and Canada belong to *C. gattii* (12). Such vast environmental reservoirs documented encourage the assumption of the existence of many other natural reservoirs of *C. neoformans*.

The infectious particles of *C. neoformans* and *C. gattii* are presumed to be the dehydrated yeast cells, which enter the alveolar spaces of the lungs. Once inside the lungs, the yeast cells become rehydrated and acquire the polysaccharide capsule which is best visible in Indian ink preparations (13). The thickness of the capsule is strain related and varies with environmental conditions and is known to be responsible for virulence (13,14). Upon growth in 1% peptone solution, production of the capsule is enhanced. *C. neoformans* can cause an asymptomatic pulmonary infection followed by the development of meningitis, which is often the first indication of the disease limited to the lungs (13).

The study was aimed at establishing environmental reservoirs for *C. neoformans* and *C. gattii* in attempt to establishing the risk factors for the high prevalence of Cryptococcal infection in Kenya. The paucity of information on the natural reservoirs of *C. neoformans* in Kenya compared to other documented information is worthwhile.

MATERIALS AND METHODS

A total of 400 samples were collected from different locations in Nairobi, (1°17'S 36°49'E / 1.283°S 36.817°E), Kenya. The city lies on the Nairobi River, in the south of the nation, and has an elevation of 1661 m (5450 ft) above sea level. Sampling was done from areas in the city with high concentrations of pigeons, birds, private chicken breeders' homes and chicken selling markets. Others were from garbage damping sites within the city, (Table 1, Figure 1). Samples were treated as described by Staib, 1987 (15). Droppings, bark and soil samples were processed as follows: 5 g of the sample were suspended in 25 ml of phosphate-buffered saline (PBS) and allowed to settle for 30 min then the sample was filtered and 50 µl of antibiotic solution, (20 U/mL streptomycin and 40 U/mL penicillin) was added. Then 100 µL of the mixture was cultured on Niger-seed agar (*Guizotia abyssinica*). All plates were incubated at 37ºC for up to two weeks. The material collected with the swabs was directly plated onto the same medium. All brown colonies were cultured on Christensen's urea agar and incubated at 37 ºC for 48 hours. Urease positive colonies were cultured on corn meal agar using slide culture technique. Each plate was divided into four parts and a single colony was seeded onto each part, it was covered with sterile cover slip and incubated at 37 ºC for 48 hours. The cover slip was removed aseptically and observed under microscope using X 40 resolution power. This was to distinguish different *Cryptococcus neoformans* from other yeasts. *Cryptococcus neoformans* do not produce pseudohyphae, are spherical, irregular in size and widely separated on corn meal agar. Observation for the presence of capsule which is a characteristic of *Cryptococcus neoformans* was done on Indian ink stain. The biovariety study was performed by culturing the isolates on Canavanine- Glycine-Bromthymol blue (CGB) medium to determine the use of glycine as a carbon source. A colour change from light yellow green to cobalt blue was considered a positive result for the CGB test, indicating presence of *C. neoformans* var. *gattii* (serotype B/C). The lack of colour change was considered a negative result, indicating absence of *C. neoformans* (serotype A/D).

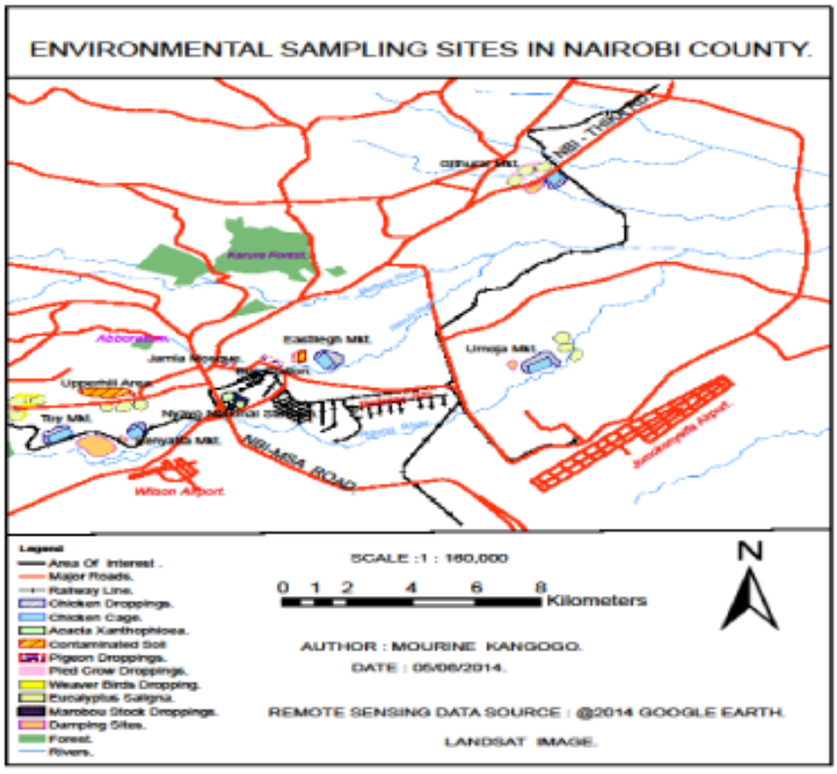
RESULTS

The environmental isolates of C. *neoformans* and *C. gattii* were recovered from different sources and sites as shown in Table 1and Figure 1. *Cryptococcus neoformans* was frequently isolated in all the droppings with the majority being from marabou stock (4/20; 20%) followed by chicken droppings (7/60; 11.7%) and 10% each from weaver birds (2/20), pigeon droppings (8/80), chicken cage (5/50) and pied crow droppings (2/20). In contrast, *Cryptococcus gattii* was less frequently isolated among the droppings with 3/80 (3.8 %) in pigeon droppings 1/20 (5%) from Marabou stock droppings and 1/60 (1.7%) from chicken droppings. Also, *C. gattii* was more frequently isolated among trees with 6/50 (12%) from *Eucalyptus saligna* and 1/10 (10%) from *Acacia xanthophloea. C. neoformans* 5/50 (10%) were recovered from *Eucalyptus saligna*. On the other hand *C. gattii* was not recovered from soil or garbage dumping sites. *C. neoformans* was isolated from soil (2/30; 6.7%) and garbage damping sites (6/60; 10%) (Table 1).

Table 1
Environmental isolates of Cryptococcus neoformans and Cryptococcus gattii in Nairobi, Kenya

Sample Type	Total Number of Samples	Positive Samples	
		Cryptococcus neoformans	*Cryptococcus gattii*
Droppings			
Marabou stock (*Leptoptilos crumeniferus*) droppings	20	4 (20%)	1 (5%)
Weaver birds (*Ploceus spekei*) droppings	20	2 (10%)	0
Pigeon (*Geopeliastriata*) droppings	80	8 (10%)	3 (3.8%)
Chicken (*Gallus gallus domesticus*) droppings	60	7 (11.7%)	1 (1.7%)
Pied crow (*Corvus albus*) droppings	20	2 (10%)	0
Tree swabs			
Acacia xanthophloea	10	0	1 (10%)
Eucalyptus saligna	50	5 (10%)	6 (12%)
Other sites			
Chicken (*Gallus gallus domesticus*) cage	50	5 (10%)	0
Garbage damping site	60	6 (10%)	0
Contaminated Soil	30	2 (6.7%)	0
Total	400	41	12

Figure 1
Map Showing Sampling Sites of Cryptococcus neoformans and C. gattii in Nairobi, Kenya



DISCUSSION

We were successful in recovering C. *neoformans* and C. *gattii* from different environmental sites in Nairobi, Kenya (Table 1 and Figure 1). This success was attributed to the fact that all the droppings analysed were dry and not wet at the time of collection. In a study conducted by Granados and Castañeda (16), it was noted that old excreta was more likely to harbour high numbers of *C. neoformans* than fresh excreta. We were also able to isolate *C. gattii* though in small percentages from pigeon droppings, marabou stock droppings and chicken droppings. C. gattii was not isolated from weaver bird and pied crow droppings. Limited studies have been carried out in Africa on the environmental isolation of *C. neoformans* (17-18). A study in Ethiopia on occurrence of *C. neoformans* in the environment showed the presence of *C. neoformans* in pigeon droppings and absence of *C. neoformans* from other bird droppings (18). The same study did not recover *C. gattii* from the pigeon droppings. In our study most of the C. neoformans isolates were recovered from pigeon (8/80; 10%) and chicken (7/60; 11.7 %) droppings. Although pigeon droppings has been documented as the most common source of *C. neoformans* in the environment, the presence of this yeast in many bird species other than pigeons, i.e., dove, psitaccines, budgerigars, canaries, parrots, cockatoos and Starlings had also been reported (19).

We were able to recover *C. neoformans* and *C. gattii* from two tree species namely *Acacia* and *Eucalyptus*. Most of the environmental *C. gattii* isolates were recovered from *Eucalyptus* trees (6/50; 12%) and only (1/10: 10%) was recovered from Acacia tree. Our findings agree with other studies that associates *C. gattii* with *Eucalyptus* and other non *Eucalyptus* trees as well (12,16). This was confirmed in a environmental surveillance study by Granados Castaneda in Bogotá, Colombia whereby C. *gattii* was recovered from *Eucalyptus* trees as well as other trees including *Acacia, Cupresus,* and *Pinus* (16). Similarly, C. *neoformans* was recovered also from *Eucalyptus* trees in this study. Many studies associate *C. gattii* isolation with *Eucalyptus* but our findings proved otherwise; the fact that these trees harbor different bird species could have contributed to the recovery of both isolates. Few reports exist of isolations of *C. neoformans* and *C. gattii* from the same habitats with the recognitions of natural hybrids between the two species. For instance, *C. neoformans* and *C. gattii* have been isolated from same sources, such as *Eucalyptus spp*. or *Syzygium cumini* trees or bird feces (20,21).

C. neoformans was isolated from other sites including soil samples, chicken cage and swabs from garbage dumping sites. Contrary to some studies that have found soil to be a major reservoir of *C. gattii* (12,22), we did not recover *C. gattii* from soil in this study.

The occurrence of *C. neoformans* and *C. gattii* in different environmental samples collected in the Nairobi, Kenya is significant. The isolation and identification of yeast from these environmental sources in Kenya might provide useful information for ecological and epidemiological studies of *C. neoformans* and *C. gattii*.

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