ENVIRONMENTAL ISOLATION OF Cryptococcus Neoformans IN JOS

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***Abstract*- Cryptococcus neoformans is an opportunistic human pathogen that causes cryptococcosis, a life-threatening infection that is usually manifested as menigeoencephalitis mainly in immunocompromised patients. The objective of this study was to evaluate the presence of Cryptococcus neoformans from four environmental sources; water, soil poultry droppings and pigeon guano in Jos, plateau state, Nigeria. Two hundred samples, fifty samples each of water, soil, poultry droppings and pigeon guano were collected from five different settlements in Jos. Each sample was suspended 1:10 in saline solution and then cultured in Sabouraud's dextrose agar medium including chloramphenicol. Identification of C. neoformans was performed on the basis of melanin synthesis on bird seed agar, presence of a capsule on India ink preparation, urease production on urea agar medium, and ability to grow at 37 °C. An assimilation test was also used to confirm C. neoformans. Of the 200 samples, 17(8.5%) were positive for C. neoformans. The highest frequency was observed in pigeon guano 8(16.0%) followed by soil 6(12.0%) and poultry droppings 3(6.0%) no isolate was made from water. The study showed the presence of C. neoformans in environmental sources especially in domestic birds.**

***IndexTerms*—Cryptococcus neoformans, pigeon droppings, isolation, immuoncompromised, Jos**

I. INTRODUCTION

Cryptococcus neoformans is a basidiomycetus yeast-like fungus.Following its first identification in nature from peach juice samples, the major environmental sources of *C. neoformans* have been found to be soil contaminated with pigeon droppings or eucalyptus trees and decaying woods forming hollows in living trees. [1]*C. neoformans* exists in two varieties; *C. neoformans var. neoformans* and C. *neoformans var. gattii*.[2] Based on antigenic determinants of polysaccharide capsule serotypes A,D and AD of *C. neoformans var. neoformans* and serotypes B and C of *C. neoformans* have been identified.[3]*C. neoformans*is an opportunistic human pathogen that causes cryptococcosis, a life threatening infection that is usually manifested as menigeoencephalitis mainly in immunocompromised patients.[4,5,6] Differences between the two varieties concerning, pathogenicity and geographical distribution has been described. *C. neoformans var. neoformans* is responsible for most cryptococcosis in immunocompromised patients, and *C. neoformans gattii* has been associated with infections in subjects with normal immunologic status.

C. neoformans var. *neoformans* has been isolated from different sources in nature and is noted for its association with accumulations of avian guano, especially pigeon excreta. It has also been isolated from droppings of caged birds including parrots, canaries, and budgerigars.[7] Other environmental isolates have been obtained from wood, rotting vegetables, soil, and dairy products. The pigeon is unlikely to be the main source of *C.neoformans* in nature because only low

concentrations of organisms are found in samples from the beak, feet, crop, and rectal swabs. [8] The internal temperature of the pigeon is 42 °C, which inhibits the multiplication of *C. neoformans*. The high concentration of ammonia in fresh droppings is also inhibitory to growth. In contrast, very high concentrations of the yeast form of the organism are found in weathered pigeon droppings, an environment that is unfavorable to the growth of most microorganisms. *C. neoformans* remains viable on dry pigeon droppings for severalyears. This can be a reservoir of persisting smallcapsules that are compatible with alveolar deposition.In many situations, reports of cryptococcosis havebeen related to pigeon droppings as the source ofinfection. However, an epidemiological analysisrevealed that patients with pigeon contact had a high exposure risk. [7] Therefore, the aim of this survey was to recover *C. neoformans* environmental isolates from water, soil, poultry droppings and pigeon droppings in Jos North LGA of Plateau state, Nigeria.

II. Materials and Methods

Sampling

Two hundred samples, fifty samples each of water, soil, poultry droppings and pigeon guano were collected from five different settlements in Jos North LGA of Plateau state, Nigeria. The droppings from pigeon were collected from cages in homes domesticating these carrier birds in some suburbs in Jos; Sabon Layi, Utan and Angwan Rimi, while poultry droppings were collected from poultry farms in the University of Jos Permanent Staff quarters and Lamingo Road. Water was collected from a flowing river within Jos Metropolis, in Jos; Dilimi stream, running through Angwan Rogo, and soil was obtained around *Eucalyptus camadulensis* within University of Jos permanent site, main Campus and Federal college of Forestry Jos, Nigeria. Samples were collected in sterile screw-capped universal containers properly labeled and taken to the laboratory for processing between the months of August and September 2008.

Isolation and sample processing

The samples were processed as follows; [7] Dry and freshly collected specimens were suspended in 5ml sterile phosphate buffered saline (PBS) and left to stand for three hours to dissolve the hard caked droppings. This was shaken vigorously and filtered through muslin gauze. The filtrate was centrifuged at 2000rpm for five minutes. Soil samples were allowed to stand in PBS for 30 minutes filtered. Water samples were centrifuged at 2000rpm for five minutes, supernatant was discarded. Aliquots of 0.5 mL supernatant were streaked on Sabouraud's dextrose agar medium (SDA) including chloramphenicol (0.05 mg/mL) with the use of an inoculation loop. The cultures were incubated at 32 °C and observed for days. Small cream off white colonies which grew on SDA was stained using lactophenol blue, coccoid cells were considered as yeast. Suspected mucoid colonies were sub-cultured onto fresh SDA and incubated at 32^{o}C for 72 hours.

Identification of *Cryptococcus neoformans* strains

Morphological and biochemical tests were used for the identification of *C. neoformans.* [9] Seventy-two hours after incubation, colonies with a mucous appetence and suspected colonies were selected. The isolates were sub-cultured to obtain single colonies on SDA plate. All isolates were identified by colony morphology and microscopic morphology of yeast cells. *C. neoformans* isolates were identified on the basis of melanin synthesis on bird seed agar, presence of a capsule on India ink preparation, ability to ferment the following; carbohydrates dextrose, galactose, urease production on urea agar medium, and ability to grow at 37°C. Inositol assimilation was also used to confirm *C. neoformans*.

Data obtained were analyzed using computerized statistical software. Statistical package for social sciences SPSS version 15, Chi-square test was used to test for significant association. P-values <0.05 were considered significant.

III. Results

C. neoformans was isolated from pigeon guano, poultry droppings and soil samples collected from five different locations selected. Out of 200 samples collected, 17(8.5%) samples were positive for *C. neoformans* (Table). The samples from pigeon guano showed the highest rate of contamination 8(16%), The samples from the soil had 6(12%) positive samples out of 50 tested samples. The samples from poultry droppings sites showed the lowest percentage of contamination 3(6%), no isolate was made from water.

Distribution of Cryptococcus neoformans based on sample source

Source	No. examined	No. isolated (%)
Water	50	0(0.0)
Soil	50	6(12.0)
Pigeon guano	50	8(16.0)
Poultry droppings	50	3(6.0)
Total	200	17(8.5)

P-values <0.05 were considered significant.

IV. Discussion

The recovery of *C. neoformans* from the pigeon excreta, poultry droppings and soil in Jos established that avian habitats and soils around Eucalyptus trees serve as an important saprobic reservoir for *C. neoformans* an opportunistic pathogen, that cause debilitating diseases among the immunocompromised. Similar studies were carried out among caged birds at Jos Wildlife Park and the zoological Garden in Jos, Plateau state Nigeria,[9] and many other researchers from different parts of the world.[10-14] The positive samples of *C. neoformans* in our study were observed in places close to dense populations, where these carrier birds are domesticated, and where the droppings have been allowed to accumulate over a long time producing phenol oxidase an essential ingredient that support the growth of the fungus. Exposure to *C. neoformans* propagules could be associated with infection risk in a given population.[3] The presence of *C. neoformans* recovered in the environment is an important finding. This fungus has been reported as an agent of opportunistic infections such as meningitis, lung infections, fungemia, abscess, and skin infection, mainly in patients with great deterioration of the immune response.[15,16] The occurrence of the agent of cryptococcosis in the areas of this study could be due to the environmental conditions favoring growth of *C. neoformans*

such as a large amount of pigeon excreta, dry excrement, and a suitable pH. Other studies have previously reported a more frequent isolation of the yeast from dry rather than from moist excrement.[6] Dry excrement is a favorable substratum since it has fewer bacteria and therefore less competition, which could help explain the higher population density found in this substratum. [17] This work reveals that (3.0 %) *C. neoformans* was isolated from the soils around eucalyptus trees. The result is statistically not significant. The source of the organism in soil could be from red gum tree eucalyptus species which seems to be the natural habitat for *C. neoformans var. gattii*. The nectar of the flowers serves as a medium for its growth. The prevalence of *C. neoformans* in Jos was high (17.0%), especially in Pigeon guano (8.0%). The cages where these birds are kept are within residential houses, in most cases in ceilings of residential houses. These practices pose a potential public health risk to the residents both immunocompetent and immunocompromised. Since the organism is transmitted basically by inhalation of pathogen when aerosols are generated by farmers, and its transportation in avian droppings and the dispersion of the yeast in the wind. , the risk of acquiring infections increases during dry season. The source of this fungus in the soil and other environmental sources could be traced to shedding from use of avian droppings by dry season. Our study shows how a pathogenic agent such as *C. neoformans* can colonize various environmental sources in a community and it is possible that this particular yeast colonizes several places by means of its transportation in avian droppings and the dispersion of the yeast in the wind.

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