RESEARCH NOTE

DETECTION OF *CRYPTOCOCCUS NEOFORMANS* IN BIRD EXCRETA

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Abstract. We evaluated 14 samples of bird excreta from pigeons, parrots, open billed storks and crows obtained from thirteen places in Bangkok and nearby areas between April and July 2004. These bird excreta were examined for *Cryptococcus neoformans* by direct plating method to inspect their ability to grow at 37ºC. Capsule production was examined by Indian ink preparation. They were also tested for urease and phenoloxidase enzymes. *Cryptococcus neoformans* var *neoformans* was recovered from pigeon excreta in 9.09%. This implies those having impaired immunity may get this fungus from the environment.

INTRODUCTION

Cryptococcus neoformans is a fungal pathogen that can cause infection in patients with immunodeficiency as opportunistic infection. Mortality from HIV-associated cryptococcal meningitis remains high (10-30%), even in developed countries, because of the inadequacy of current antifungal drugs and the complication of increased intracranial pressure (Bicanic and Harrison, 2005). HIV-infected children have also developed cryptococcosis. The 8-year point prevalence of cryptococcosis among hospitalized HIV-infected patients was found in one study to be 2.97% (Likasitwattanakul *et al,* 2004). Cryptococcosis is not only found in those HIV-infected children but also in those with systemic lupus erythematosus (Pancharoen *et al,* 2001). Non-HIV-related cryptococcosis has also been reported, including cutaneous cryptococcosis, pulmonary cryptococcosis and cryptococcal infection of the central nervous system (CNS) (Yao *et al,* 2005). Chierakul *et al* (2005) found that *Cryptococcus neoforman* was among other organisms with increased infection rates in HIV-infected patients and is the commonest condition requiring admission due to cryptococcal meningitis (15%) (Inverarity *et al*, 2002). Its reservoir is pigeons which come into close contact with people both healthy and those with immunodeficiency, because their eating places are limited. Many people enjoy feeding pigeons which is another way of bringing them close together. The AIDS epidemic has shown the importance of studying the epidemiology of this fungus. Thus we conducted a survey of the prevalence of *C. neoformans* in pigeons, crows, open billed storks and parrots.

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MATERIALS AND METHODS

Thirteen locations were chosen for this

Table 1
Positive findings for *Cryptococcus neoformans* in 13 areas from 14 pooled samples of bird excreta.

Kind of Birds	Total number of samples	Total number of positive	Percent of positive
Pigeon	11	1	9.09
Crow	1	0	0
Open billed stork	1	0	0
Parrot	1	0	0

study. Pooled excreta were collected in each setting. Twelve areas were public areas; 11 of the 12 areas were pigeon areas, 1 was for crows, 1 was for open billed storks and another setting was a closed system farm for parrots.

Detection of *Cryptococcus neoformans*

The method performed has been described by Pal (1997) with some modifications. Ten grams of each specimen was suspended in a sterilized glass bottle containing 90 ml of sterile physiologic saline (NSS). The solution was filtered through sterile gauze cloth. The filtrate was left at room temperature for about 10 minutes. One ml of supernatant was transferred to another sterile bottle which contained 9 ml of sterile NSS, then shaken manually for 20-30 seconds. The solution was streaked onto Sabouraud's dextrose agar containing 0.05 mg/ml of chloramphenicol. The medium was then incubated at 37ºC for 48-72 hours. Round colonies with a smooth surface and cream-colored were then transferred to Pal's medium. The medium was incubated at 37ºC and examined daily for 7 days. The colonies which grew on this medium and were dark brown pigmented were stained with Indian ink. A urease test was performed and the production of phenoloxidase enzyme on GGA-DOPA medium was determined. The D-proline assimilation test was done as described by Dufait *et al* (1987). Colonies which were urease positive and phenoloxidase enzyme positive were considered as *C. neoformans.*

RESULTS

Of the 14 samples from thirteen areas examined for *Cryptococcus neoformans.* The fungus was found in only 1 sample (9.09%) of pigeon excreta, recovered on both Sabouraud's dextrose agar and Pal's medium.The assimilation test confirmed the fungus was *C. neoformans* var *neoformans.* The rest of the bird excreta were negative, as shown in Table 1.

DISCUSSION

The recovery of *Cryptococcus neoformans* from pigeon excreta emphasis this opportunistic pathogen exists in the environment. Our study found the incidence of this fungus at 9.09 %, while in Nepal it was found in 25% of samples (Pal, 1977).

Cryptococcal meningitis is a common opportunistic infection in AIDS patients, particularly in Southeast Asia and Africa. Cryptococcosis accounts for 13-44% of all deaths in AIDS (Bicanic and Harrison, 2005). Cases also occur in patients with other forms of immunodeficiency and in apparently immunocompetent individuals. Cryptococcal antigen in the cerebrospinal fluid of those with cryptococcal meningitis has been found positive on all tested samples and Indian ink preparations were positive in 94% of cases. However, routine CSF examinations are normal in 50% of cases (Likasitwattanakul *et al,* 2004) which can result in a missed diagnosis. Amphotericin B,

flucytosine, fluconazole and itraconazole have been used effectively to treat this infection (Imwidthaya and Poungvarin, 2000).

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Transfusion Medicine and the Faculty of Allied Health Sciences, Chulalongkorn University for supporting this research.

REFERENCES

Bicanic T, Harrison TS. Cryptococcal meningitis. *Br Med Bull* 2005; 72: 99-118.

Chierakul W, Rajanuwong A, Wuthiekanun V, *et al.* The changing pattern of bloodstream infections associated with the rise in HIV prevalence in northeastern Thailand. *Trans R Soc Trop Med Hyg* 2004; 98: 678-6.

Dufait R, Velho R, de Vroey C. Rapid identification of the two varieties of *Cryptococcus neoformans* by D-proline assimilation. *Mycoses* 1987; 30: 483.

Inverarity D, Bradshaw Q, Wright P, Grant A. The spectrum of HIV-related disease in rural Central Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 822-31.

Imwidthaya P, Poungvarin N. Cryptococcosis in AIDS. *Postgrad Med J* 2000; 76: 85-8.

Likasitwattanakul S, Poneprasert B, Sirisanthana V. Cryptococcosis in HIV-infected children. *Southeast Asian J Trop Med Public Health* 2004; 35: 935-9.

Pancharoen C, Chindamporn A, Thisyakorn U. Childhood cryptococcosis: an increasing problem in the era of AIDS. *J Med Assoc Thai* 2001; 84 (suppl 1): S86-90.

Pal M. First report of isolation of *Cryptococcus neoformans* var. *neoformans* from avian excreta in Kathmandu, Nepal. *Rev Iberoam Micol* 1997; 14: 181-3.

Yao Z, Liao W, Chen R. Management of cryptococcosis in non-HIV-related patients. *Med Mycol* 2005; 43: 245-51.