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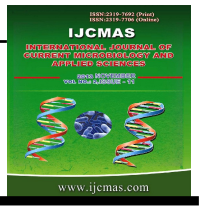

Original Research Article

Isolation and Identification of *Cryptococcus neoformans* from pigeon droppings in Tiruchirappalli district of Tamil Nadu, South India

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A B S T R A C T

Cryptococcus has been a significant cause of morbidity and mortality in patients with Aids. Many reservoirs of the agent *Cryptococcus neoformans* have been reported, but the ecology of this yeast must be elucidated in order to establish surveillance programs and to prevent infections. The objective of the study was to evaluate the presence of *Cryptococcus neoformans* in pigeon droppings. Thirty three samples of pigeon droppings were collected from 10 different regions in Tiruchirappalli district. Of the thirty three samples, 20 (60.6%) were positive for *Cryptococcus neoformans*. The highest frequency was observed in droppings from site 1(100%), 3(80%), 5(80%). The lowest frequency was observed in 2(57%), 4(60%), 8(50%), 9(50%), 10(50%). The samples from the sites 6, 7 did not show any contamination to *Cryptococcus neoformans*.

K e y w o r d s

Cryptococcus neoformans; pigeon droppings; frequency; Tiruchirappalli district.

Introduction

Cryptococcus neoformans is encapsulated basidiomycetous yeast, which can cause life threatening meningoencephalitis in immunocompromised individuals, especially those with AIDS (Mattson *et al*., 1999). *Cryptococcus neoformans* was divided into three varieties; *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var.*gattii* (serotypes B and C) (Franzot *et al.,* 1999). *Cryptococcus neoformans* has a world wide distribution and has been associated with a variety of environmental sources in particular, birds excreta and decaying wood (Walter et al., 1968; Ruiz *et al*., 1981). The association between *Cryptococcus neoformans* and birds excreta, especially weathered pigeon droppings, has been confirmed in several studies.

Cryptococcosis has been responsible for great morbidity and mortality rates among patients with aids (Fernandez *et al*., 2000; Rozenbaum *et al*., 1990; Ruiz *et al*., 1982), considered the fourth most common infection in the immunocompromised individuals

(Hubalek et al., 1989). The majority of *Cryptococcus neoformans* isolates recovered from HIV positive patients has been serotype A and a similar prevalence has been found among patients that have developed the acquired immuno deficiency syndrome (Bottone *et al*., 1987; Mitchell and Perfect, 1995; Rozenbaum *et al*., 1990; Rozenbaum and Goncalves, 1994; Silva and Paula, 1963). In many situations, reports of Cryptococcosis have been associated to pigeon droppings as the source of infection; however, an epidemiological analysis showed that patients with pigeon contact had a high exposure risk (Litman and Borok, 1968). The major problem is that *Cryptococcus neoformans* remains viable on dry pigeon droppings, for many years, being a reservoir of inhaled infecting particle, persisting with small capsule which are compatible with alveolar deposition. The most important natural sources of *Cryptococcus neoformans* var. neoformans throughout the world is pigeon excreta and soil contaminated with avian droppings (Swine, 1975; Schneider and Stenderup, 1982) whereas the natural habitat of *Cryptococcus neoformans* var. gatti in several species of Eucalyptus trees (Ellis and Pfeiffer, 1990). In this study, we aimed to verify the presence of *Cryptococcus neoformans* in pigeon dropping.

Materials and Methods

Sampling

Thirty three samples of pigeon droppings were collected from 10 various sites in Tiruchirappalli. Pigeon excreta samples were collected using spatulas, transferred to clean plastic bags, and properly labeled according to sites and date. The average sample weight was around 500g. Samples were taken to the laboratory and were used immediately. The number and the types of samples collected at different sites are shown in Table -1.

Isolation and sample processing

The samples were processed according to Casali et al., 2003 1g of weathered pigeon excreta were added to 10 ml of sterilized saline solution (0.9%) with chloromphenicol at 150mg ml^{-1} achieving 1:10 dilution. After filtration trough gauze, aliquots of 10^{-1} dilutions (birds excreta) were inoculated on to potato dextrose agar plate, and incubated at 37 □ C. The contamination of each sample was confirmed by the existence of *Cryptococcus neoformans* in it. The highest and lowest contamination with *Cryptococcus neoformans* in the studied zones was explained with the percentage of positive samples in each site.

Identification of *Cryptococcus neoformans* strains

Morphological tests were used for the identification of *Cryptococcus neoformans.* Seventy two hours after incubation, colonies with a mucous appetence and suspected colonies were selected. All isolates were identified by colony morphology and microscopic morphology of yeast cells. *Cryptococcus neoformans* isolates were identified on the basis of presence of a capsule on India ink preparation.

Results and Discussion

Cryptococcus neoformans was isolated from pigeon dropping samples collected from 8 out of 10 sites selected. Out of 33 samples collected, 21 (63.6%) samples were positive for *Cryptococcus neoformans*. The samples from site 1 showed the highest rate of contamination

(100%), 3 positive samples out of 3 tested samples. The samples from sites 8,9,10 showed the lowest percentage of contamination (50% each). Only one positive sample was obtained from each of these sites. Only one sample was obtained from the site 6, 7. These two sites did not show any contamination to *Cryptococcus neoformans*. The samples from the sites 3, 5 showed the moderate rate of contamination (80%) 4 positive samples out of 5 tested samples. The samples from the sites 2,4 showed the average rate of contamination (57%,60%). The positive samples of *Cryptococcus neoformans* in our study were observed in places close to dense populations. Pigeon droppings have been reported as important substrates for the presence and maintenance of *Cryptococcus neoformans* in the environment. The most important reason for the high frequency of *Cryptococcus neoformans* in the collected zones could be environmental conditions such as heavy plant growth, large amount of pigeon droppings, and transfer of *Cryptococcus neoformans* in zones by pigeons.

Absence or low contamination in some zones could be due to small number of trees and pigeon lofts in the zones. Pigeon droppings have been reported as the major environmental source of *Cryptococcus neoformans* in several countries (Yehia, 1999; Kiel stein *et al*., 2000; Yimtubezenash *et al*., 2001), but in India, there have been very limited studies on the occurrence and identification of the *Cryptococcus neoformans* in public areas. Therefore, the isolation and identification of yeast from pigeon droppings in India might provide useful information for ecological and epidemiological studies of *Cryptococcus neoformans.* In the present study, the potential health hazard posed by the occurrence and identification of *Cryptococcus neoformans in* pigeon droppings were investigated in samples collected from public areas in Tiruchirappalli.

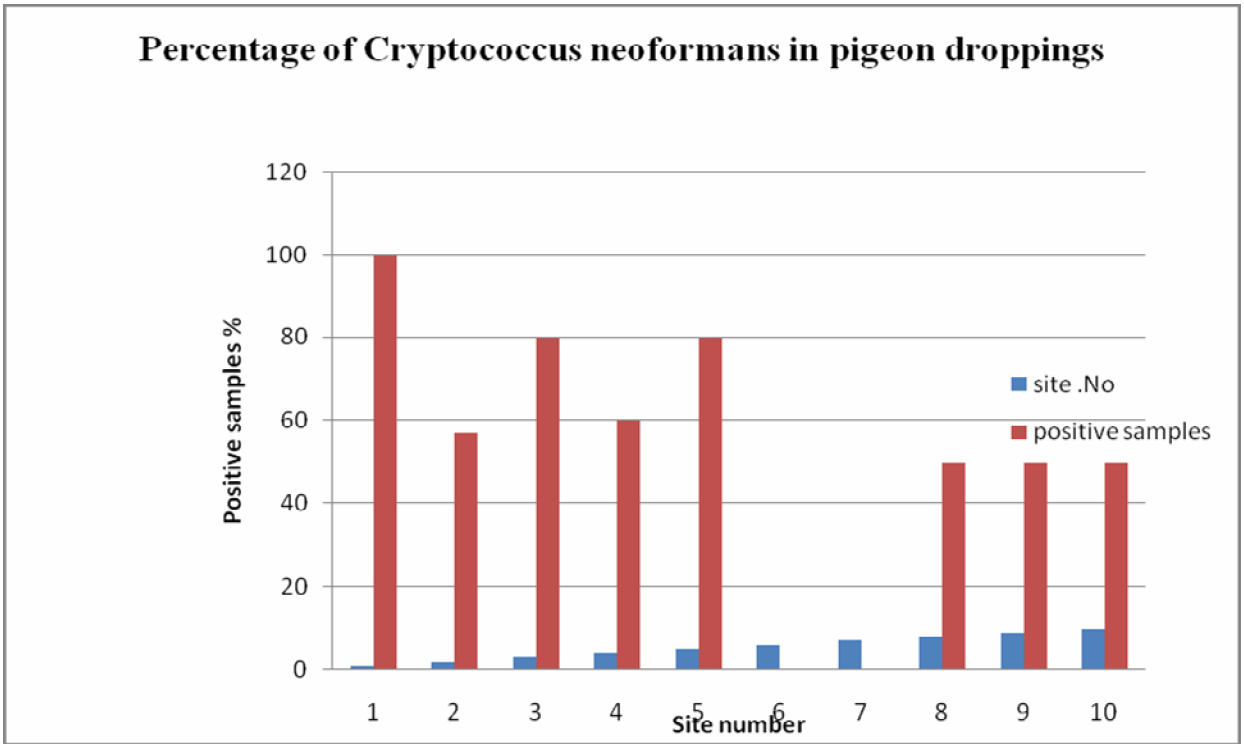
All the isolates of *Cryptococcus neoformans* used in this study were obtained from old withered pigeon droppings, not fresh droppings, which was in accordance with the previous reports that *Cryptococcus neoformans* was not found from fresh pigeon droppings samples (Mishra *et al*., 1981). Emmons (1995) established a frequent saprobic association of *Cryptococcus neoformans* with the old excreta and nest of pigeons. Yamamoto *et al*., (1995) reported *Cryptococcus neoformans* was isolated from 4 of 8 samples (50%) of pigeon excreta from Nagasaki. Paul (1997) reported that the prevalence of *Cryptococcus neoformans* from pigeon droppings was 7 of 28 samples (25%) in Kathmandu. Casadevall and Perfect (1998) reported that *Cryptococcus neoformans* may not be a natural inhabitant of fresh pigeon droppings. Rather, pigeon droppings might be inoculated by cells of *Cryptococcus neoformans* from environmental sources such as contaminated soil, or air, as they provide a good source for the growth of yeast. Chee and Kim (2003) in his study, the occurrence of *Cryptococcus neoformans* was investigated in pigeon droppings collected from only three localities in Seoul. In the present study, thirty three samples were collected from 10 different sites in Tiruchirappalli district. Chee and Kyung (2005) reported that 72 pigeon dropping samples were collected from 26 different localities in Seoul and investigated for the occurrence of *Cryptococcus neoformans*. All the isolates belong to *Cryptococcus neoformans* var. grubby (serotype A).

Table.1 Distribution of *C. neoformans* isolated from pigeon Droppings in Tiruchirappalli district

Site number	N	Positive samples	
		n	%
1.	3	3	100
2.	7	4	57
3.	5	4	80
4.	5	3	60
5.	5	4	80
6.	1	0	0
7.	1	0	0
8.	2	1	50
9.	2	1	50
10.	2	1	50
Total	33	21	52.7

N: total number; n: number of positive samples

Figure.1



In Thailand, the incidence of *Cryptococcus neoformans* from chicken feces in suburban areas was 24.0% (Kuroki et al., 2004). Kwang and Hwang (2005) reported that the *Cryptococcus neoformans* were recovered from environmental sources, out of the 29 environmental samples (41.7%) of pigeon droppings especially from pigeon shelters. Rosario (2008) reported from Spain that pigeons were not the only reservoir of *Cryptococcus* and other birds could act as reservoir for it too (Rosario, 2008). In 2008, Lugarini from Brazil reported isolation of *Cryptococcus neoformans* from feces of parrots and sparrows. He stated that feces of domestic birds and those around as act as reservoir for *Cryptococcus neoformans* (Lugarini *et al*., 2008). In 2009, Souza isolated *C. gatti*, serotype B from feces of pigeons and stated that 99% of *Cryptococcus neoformans* isolates he evaluated in his study were a serotype of *grubii* variety (Liaw, 2010).

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