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Isolation of *Cryptococcus neoformans* from pigeon droppings in Ahwaz, Iran

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Aim: *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* in Ahwaz, Iran.

Materials and methods: Sixty-five samples of pigeon droppings were collected from 10 different regions in Ahwaz. 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*.

Results: Of the 65 samples, 22 (34%) were positive for *C. neoformans*. The highest frequency was observed in droppings from site 7 (86%). The lowest frequency was obtained on samples from sites 2, 3, and 4 (17%).

Conclusion: Our study showed the presence of *C. neoformans* in urban environmental sources at places with a large population in Ahwaz.

Key words: *Cryptococcus neoformans*, pigeon droppings, isolation, Iran

Introduction

Cryptococcus neoformans is an opportunistic human pathogen that causes cryptococcosis, a life-threatening infection that is usually manifested as menigeoencephalitis mainly in immunocompromised patients (1-3).

C. neoformans is a basidiomycetous, yeast-like fungus that, following inhalation from an environmental source, causes respiratory and neurological infection in humans and animals. This fungus has 5 serotypes (A, B, C, D, and AD), and recently was subdivided into 3 varieties known as *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *neoformans* (serotype D), and *C. neoformans* var. *gattii* (serotype B and C) (4). According to this classification, the correct designations for serotype AD isolates were not yet resolved.

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. Other

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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 (5). 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 several years. This can be a reservoir of persisting small capsules that are compatible with alveolar deposition. In many situations, reports of cryptococcosis have been related to pigeon droppings as the source of infection. However, an epidemiological analysis revealed that patients with pigeon contact had a high exposure risk (5). Therefore, the aim of this survey was to recover *C. neoformans* environmental isolates from pigeon droppings in different urban areas in Ahwaz, Iran.

Materials and methods

Sampling

Sixty-five samples of pigeon droppings were collected from 10 various regions in Ahwaz. Iran, over of a period of 1 year (2007).

Pigeon excreta samples were collected using spatulas, transferred to clean plastic bags, and properly labeled according to site and date. The average sample weight was around 500 g. Samples were taken to the laboratory and were used immediately. The number and the type of samples collected at different sites are shown in Table 1.

Isolation and sample processing

The samples were processed according to Casali et al. (6). A portion of excreta (about 20-30 g) from each sterile plastic bag was aseptically removed, weighed, and transferred to Erlenmeyer flasks containing a saline solution (0.9%) with chloramphenicol (200 mg/L), achieving 1:10 dilution (w/v). The material was homogenized by shaking and allowed to stand for 30 min. Aliquots of 0.5 mL supernatant were streaked on Sabouraud's dextrose agar medium (Merck) including chloramphenicol (200 mg/L) with the use of an inoculation loop. The cultures were incubated at 32 °C and observed daily for 15 days. The contamination of each sample was confirmed by the existence of *C. neoformans* in it. The highest and lowest contamination with *C. neoformans* in the studied zones was explained with the percentage of positive samples in each site.

Identification of *Cryptococcus neoformans* strains

Morphological and biochemical tests were used for the identification of *C. neoformans* (7). Seventy-two hours after incubation, colonies with a mucous appetence and suspected colonies were selected. The isolates were subcultured to obtain single colonies on Sabouraud's dextrose agar 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, urease production on urea agar medium, and ability to grow at 37 °C. Inositol assimilation was also used to confirm *C. neoformans*.

Results

C. neoformans was isolated from pigeon dropping samples collected from 8 out of 10 sites selected. Out of 65 samples collected, 22 (34%) samples were positive for *C. neoformans* (Table).

The samples from site 7 showed the highest rate of contamination (86%), 6 positive samples out of 7 tested samples. The samples from sites 2, 3, and 4 showed the lowest percentage of contamination (17% each). Only 1 positive sample was obtained from each of these sites. These sites are situated in the center and south-east of Ahwaz. Two sites did not show any contamination to *C. neoformans*.

Discussion

The recovery of *C. neoformans* from the pigeon excreta in Ahwaz established that avian habitats serve as an important saprobic reservoir for this opportunistic pathogen. This finding has already been confirmed by many researchers from different regions of the world (8-12). The positive samples of *C.*

Table. Distribution of *C. neoformans* isolated from pigeon droppings in Ahwaz, Iran.

Site number	N	Positive samples	
		n	(%)
1	11	3	27
2	6	1	17
3	6	1	17
4	6	1	17
5	5	4	80
6	7	2	29
7	7	6	86
8	7	0	0
9	6	4	67
10	4	0	0
Total	65	22	34

N: total number n: number of positive samples.

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 *C. neoformans* in the environment. The exposure to isolates of *C. neoformans* could be associated with the infection risk in a given population (1).

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 (13,14).

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 (15). 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 (15).

In our research, a heavy contamination of *C. neoformans* was presented in the 3 places studied. These zones are close to parks with many trees and pigeon lofts. The most important reasons for the high frequency of *C. neoformans* in these zones could be environmental conditions such as heavy plant growth, large amounts of pigeon droppings, and transfer of *C. 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. Although the material was sufficient in places 8 and 10 *C. neoformans* was not observed. We were successful in recovering *C. neoformans* in 34% of the samples of pigeon droppings on bird seed agar. The development of brown colonies on bird seed agar within 3-4 days at 30 °C resulted in the rapid isolation and quick presumptive identification of the yeast.

Our study shows how a pathogenic agent such as *C. neoformans* can colonize variously in the different zones of a city and how its population densities can be carried within the zones. 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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