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Research Article

PREVALENCE OF CRYPTOCOCCUS NEOFORMANS IN DRY DROPPINGS OF DOMESTIC PIGEONS IN CHANDIGARH

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A B S T R A C T

Pigeon droppings are potential environmental source of yeast *Cryptococcus neoformans* which, if inhaled may lead to a life threatening disease known as cryptococcosis. Since Chandigarh is rich in flora and fauna with abundance of pigeons, it is important to assess the prevalence of cryptococcus in pigeon droppings of this region. Sixty-one samples of dried and old pigeon droppings were collected from different sectors in Chandigarh. Using standard culture techniques, 37.7% of the samples (23/61) were found to be positive for the genus Cryptococcus spp. comprising of *C. neoformans* 17 (27.87%), *C. gatti* 4 (6.56%), *C. laurentii* 1 (1.64%). One (1.64%) sample was positive for both *C. neoformans* and *C. gattii*. With a moderate prevalence in city, the yeast positivity surprisingly was maximum in droppings collected from hospital area (62.5%). Hospital areas therefore, should be a priority for pigeon control authorities to prevent infection spread.

INTRODUCTION

Cryptococcus neoformans (C. neoformans) is an encapsulated yeast that survives both in plants and in animals. When humans inhale the infectious propagules of this fungus, which are present in the environment it leads to a life threatening disease known as cryptococcosis. A high incidence of cryptococcal meningitis has been reported in immunocompromised patients particularly from developing countries.1 Since the prevalence of HIV/AIDS is quite high in India, the country documents a high prevalence (1.7-4.7%) of cryptococcosis in these patients.2-4 Besides occurring in immunocompromised patients, sometimes it may also affect immunocompetent individuals as 10-40% of patients with cryptococcosis are apparently immunocompetent.5,6

C. neoformans belongs to genus cryptococcus, which includes over 37 species of encapsulated yeast. Of these *C. neoformans* and *C. gattii* are considered to be the most pathogenic while *C. laurentii* and *C. albidus* occasionally may cause moderate to severe disease particularly meningitis. Biological reservoirs of *C. neoformans* include soil, surface of eucalyptus and other tropical trees, plant products e.g. fruit, vegetables etc. and bird excreta. Pigeons probably serve as the most important reservoir for *C. neoformans*.7-9

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Being rich in flora and fauna, Chandigarh is an ideal habitat for all kinds of avian fauna including domestic and migratory birds. Pigeons are seen in quite abundance in Chandigarh and are frequently seen roosting and soiling at every place including residential areas, making large population susceptible to cryptococcal infection. Hence, it is important to evaluate the pigeon droppings in this region as an environmental source of pathogenic yeast Cryptococcus. This would help in assessing the risk of cryptococcal infection and acting appropriately, if required, to avoid or prevent exposure to susceptible population and to limit the spread of infection.

MATERIALS AND METHODS

Study Area

Chandigarh, the capital of Punjab and Haryana states is located in northern part of India. It covers an area of approximately 114 km and its population is estimated to be over 1 million as per 2011 India census. It has a humid subtropical climate and is characterized by a seasonal rhythm like very hot summers, mild winters, unreliable rainfall and great variation in temperature.10

Different sectors and places (40 different locations) of the Chandigarh were visited and areas with heavy pigeon's inhabitation preferably residential, hospital, holy places, commercial complexes, and bus and railway station were selected.

Sample Collection

The sample collection was carried out in non-rainy season. Sixty-one samples, each containing about 10-15 grams of dried and old pigeon droppings from shaded areas were collected using wooden spatulas and placed in sterile screw capped universal containers.

Isolation and sample processing

The specimen from each site was suspended in a concentration of 1gm/100ml sterile physiological saline (0.85% NaCl) supplemented with chloramphenicol (10 mg/ml). The material was homogenized by shaking at 200 rpm at 37°C for 2 hours and allowed to stand for 30 min. Tenfold serial dilutions were made and 0.1 ml of each dilution streaked onto duplicate plates of Bird seed agar (BSA; *Guizotia abyssincia* seeds, creatinine, dextrose, agar, chloramphenicol, biphenyl and agar) and kept at 37°C for 2-7 days. Each inoculated plate examined daily for yeast growth. After 5 days of incubation smaller brown pigmented mucoid colonies appeared on agar (Figure 1a).

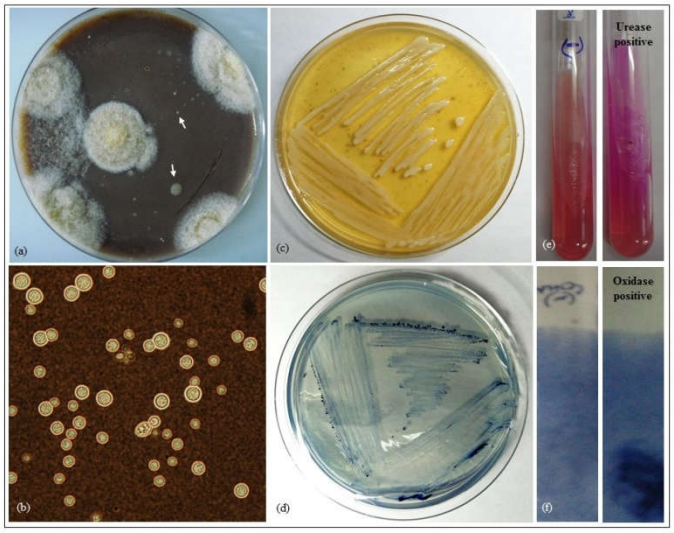

Figure 1 Culture and biochemical analysis for identification of Cryptococcus spp. (a) Bird seed agar on 5th day of incubation showing smaller brown pigmented mucoid colonies (arrow). (b) India ink preparation showing encapsulated yeast indicated by clear zones around the cells. (c) Dark cream colored, smooth, moist, shining and mucoid colonies on Sabouraud's dextrose agar. (d) Cryptococcus Differential Agar showed light blue dry colonies of *C. neoformans* after 7 days of incubation. (e) Christensen slant changing in to pink colour suggesting urease production by the *C. neoformans.* (f) Oxidase positive yeast producing bluish purple colour on paper strips.

Identification of Cryptococcus spp

For morphological identification of Cryptococcus spp., suspected colony picked up from BSA and mixed with a drop of Indian ink on a clean glass slide. After placing cover slip, the slides were examined under the microscope for presence of encapsulated yeast indicated by clear zones (negative staining) around the cells (Figure 1b). The Indian ink positive colony was sub cultured on Sabouraud's dextrose agar (SDA; dextrose, mycological peptone and agar with 0.05 mg/ml chloramphenicol) which showed dark cream colored, smooth, moist, shining and mucoid colonies (Figure 1c). The same colony was also streaked on Cryptococcus differential agar (CDA; glucose, glycine, DL-Tryptophan, thiamine hydrochloride, potassium dihydrogen phosphate, trypan blue, magnesium sulphate and agar) for differentiating *C. neoformans, C. gatti* and *C. laurentii*. After 5-6 days of incubation at 30°C, the *C. neoformans, C. gatti and C. laurentii* showed light blue dry (Figure 1d), brown mucoid and brown dry colonies respectively.

After 36-48 hours of incubation at 37°C, the pure colony was further confirmed by biochemical investigations (phenol oxidase and urease test). Urease catalyzes the hydrolysis of urea to ammonia and carbamate. *C. neoformans* produces large amount of urease. The urease test was carried out by inoculating a colony of *C. neoformans* on urea agar base, Christensen slant containing 40% urea. After 8-12 hours of incubation at 37°C, the colour of the medium was observed and a pink colour was produced by positive culture (Figure 1e).

C. neoformans is unique in its oxidase activity on o- and p-diphenols. The colonies of *C. neoformans* were rubbed with glass rod on 1% w/v NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride soaked paper stripes. The positive culture developed a bluish purple colour within few seconds (Figure 1f). Isolates were confirmed as *C. neoformans* once all tests were found to be positive.

RESULTS

Of the 61 samples, 23(37.7%) were positive for the yeast *Cryptococcus.* Among these 27.9% of samples were positive for *C. neoformans,* 6.6% for *C. gatti,* 1.6% for *C. laurentii* and 1.6% for both *C. neoformans* and *C. gattii*. Maximum prevalence of *C. neoformans* was found in samples collected from hospital (62.5%) and residential area (33.3%) followed by commercial complexes (24.3%) and bus or railway stations (20%). Out of eight dropping samples from hospital area one (12.5%) was positive for *C. gattii* and one (12.5%) for both *C. neoformans* and *C. gattii*. Two of six samples from residential area showed *C. neoformans.* Five samples each were collected from holy places and Bus/railway station. Only one (from railway station, 20.0%) was found to be positive for *C. neoformans.* Of the thirty-seven samples collected from commercial complexes three (8.10%) were positive for *C. gattii* and one (1.64%) for *C. laurentii*. Holy places were all free of Cryptococcus yeast. C. *gattii* and C. *laurentii* were found only in hospital area and commercial complexes.

Based on culture (BSA and CDA), Indian ink staining and biochemical (Oxidase and urease) characterisation, the study demonstrated the maximum prevalence of *C. neoformans* (75%) followed by *C. gattii* (20.83%) and *C. laurentii* (4.16%) (Figure: 2).

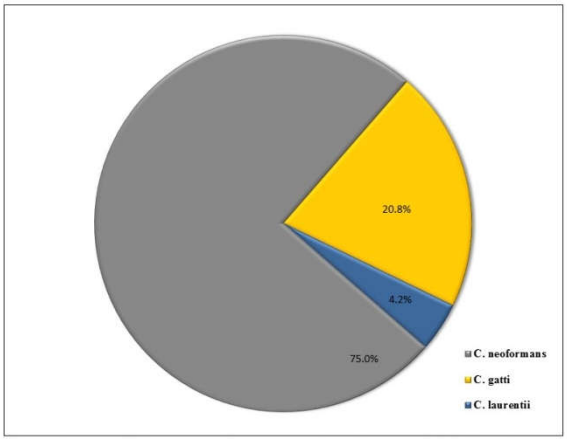

Figure 2 Percentage of different species of Cryptococcus found in positive samples (24/61) of pigeon droppings.

DISCUSSION

Two varieties of *C. neoformans: C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* are commonly associated with disease in humans. In the present study, 95.8% of positive samples harboured either *C. neoformans* var. *neoformans* or *C. neoformans* var. *gattii.* Both of these, particularly *C. neoformans* var. *gatti*, are capable of causing cryptococcosis in immunocompetent individuals.11 Hence accumulates of pigeon droppings in and around the hospital, residential areas and commercial complexes are potential source of cryptococcal infection not only for immunodeficient or hospitalized patients but also to the general population.

Our results show a prevalence rate of 37.7% for the Cryptococcus yeast in pigeon droppings. Despite rich flora and fauna of Chandigarh, the figure fortunately is lesser than some other regions of India (table).12-16 The probable reasons could be differences in the flora of this reason and awareness about hygiene and cleanliness among the resident population.

Table Prevalence of Cryptococcus in pigeon droppings reported from India

Prevalence rate	Samples analysed	Place of sample collection	Geographical area	References
67.0%	21	Various sites including hospital area	Vellore	Abraham et al.[12]
60.6%	33	Various sites	Tiruchirappalli district of Tamil Nadu	Xavier et al.[13]
57.9%	69	Bird Hospital, University campus	Northern Delhi	Khan et al.[14]
10.0%	149	Various sites	Delhi, Haryana, Uttar Pradesh and Rajasthan	Gugnani et al.[15]
6.2%	32	Institute building	Northern Delhi	Sethi et al[16]
37.7%	61	Various sites including hospital area	Chandigarh	Present study

Gugnani *et al*15 and Sethi *et al*16 reported even lesser prevalence rate (10.0% and 6.2% respectively) in their study. However, the reason for their low prevalence could be the site of collection of pigeon droppings. In both the studies droppings coming in direct contact with sunlight were also collected. It has been shown previously that direct sunlight has deleterious effects on the viability of Cryptococcus while it can be easily recovered from pigeon excreta in sheltered than from the open sites.17 In our study most of the droppings were old and dried and collected from sheltering sites rather than the sun exposed areas.

Similar to our findings, worldwide literature strongly established that pigeon's droppings are major source of *C. neoformans*. Rosario *et al* studied 331 pigeon cloacal swabs and reported 26 (7.85%) swabs harbouring Cryptococcus spp. out of which 3.32%, 1.81%, 1.81% and 0.91% samples were positive for *C. uniguttulatus, C. laurentii, C. neoformans var. neoformans* and *C. albidus* respectively. The results show importance of pigeons in the cryptococcosis epidemiology as a reservoir and carrier of *C. neoformans var. neoformans* along with other Cryptococcus spp. of clinical interest.14 In another study, Rosario *et al* in Grand Canary Island isolated Cryptococcus spp. in 47.13% of pigeon's droppings of which *C. neoformans* was 33.9% and *C. laurentii* was 4.59%.15 Teodoro *et al* recovered Cryptococcus spp. in 66.6% of 87 bird's droppings from different locations in the City of Araraquara out of which *C. neoformans* were 17.2%, *C. gattii* 5.2%, *C. laurentti* 1.7% and *C. luteolus* (1.7%). High positivity was recorded in hospital area or in close proximity to hospitals.16 Recently, in a Nigerian study on 177 pigeon droppings obtained from 10 different cities, *C. neoformans* was isolated in 32 (22.0%) of samples of which 32.4% were recovered from dovecotes, 31.8% from market and 4.0% from churches. Surprisingly *C. gattii* was not found in any of the samples.17 In contrast to previously reported findings the present study shows higher prevalence (62.5%) of *C. neoformans* in hospital area followed by residential area (33.33%), commercial complexes (24.32%) and bus or railway station (20%).

Cryptococcosis due to inhalation of yeast in hospital area is frequently reported in India. Gupta *et al* reported cryptococcosis in 1.9% of patients who had underwent renal transplantation in our institute.18 Dharwadkar *et al* found cryptococcus in bone marrow of a 40-year-old female with HIV infection.19 Gunani *et al* isolated *C. neoformans* in pigeon droppings from Delhi Zoological Park (India) in addition to the sputum samples of park's employees who had chest pain, low grade fever, mild cough and mucoid expectoration at the time of investigation.20 Yamamoto *et al* isolated *C. neoformans* from 4 of 8 pigeon excreta samples (50%) in hospitals, private houses, parks in Nagasaki and during this study they found two pulmonary cryptococcosis patients in the area where *C. neoformans* was isolated.21 All these observation suggests a possible link between pigeon droppings and cryptococcosis with higher risk in and around hospitals as there are several patients who are immunocompromised and susceptible. Therefore, more attention and rigorous control measures are required especially in hospital areas to prevent the spread of infection.

CONCLUSION

Study suggests that occurrence of *C. neoformans* along with other yeasts in hospitals; residential, commercial places and bus or railway stations might be potential threat to population especially immunocompromised patients of Chandigarh. Because Chandigarh is the major health care center for North-Indian population, a higher prevalence of pigeon droppings harboring *C. neoformans* around the hospital and nearby residential area could be a threat to already sick individuals visiting to hospital as well as to nearby residents.

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Conflict of Interest

None

Ethical Approval

Not required

References

1. Park BJ, Wannemuehler KA, Marston BJ, *et al*. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009; 23:525-530.
2. UNAIDS. Country progress reports - 2015 [Online] Available at: http://www.unaids.org/en/ regionscountries/countries/india/
3. Kumarasamy N, Solomon S, Flanigan TP, *et al*. Natural history of human immunodeficiency virus disease in southern India. *Clin Infect Dis.* 2003; 36:79-85.
4. Vajpayee M, Kanswal S, Seth P, *et al*. Spectrum of opportunistic infections and profile of CD4+ counts among AIDS patients in North India. Infection. 2003; 31:336-340.
5. Pappas PG, Perfect JR, Cloud GA, *et al*. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis*. 2001; 33:690-699.
6. Lui G, Lee N, Ip M, *et al*. Cryptococcosis in apparently immunocompetent patients. *QJM*. 2006; 99(3):143-151.
7. Rosario L, Soro G, Deniz S, *et al*. Presence of C. albidus, C. laurentii and C. uniguttulatus in crop and droppings of pigeon lofts (Columbia livia). *Mycopathologia*. 2010;169(4):315-319.
8. Ior CA, Ayanbimpe G, Ior LD, *et al*. Environmental isolation of Cryptococcus neoformans in JOS. *Sci Res J*. 2015;3(6):15-27.
9. Maryam S, Mansour B, Hashemi SJ, Mohammadali Z, Nader P. Isolation of Cryptococcus neoformans and other opportunistic fungi from pigeon droppings. *J Res Med Sci.* 2013;18(1):56-60.
10. Provisional Population Totals, Census of India 2011; Urban Agglomerations/Cities having population 1 lakh and above. Office of the Registrar General & Census Commissioner, India. Available at:
11. http://www.censusindia.gov.in/2011-prov-results/paper2/prov_results_paper2_indiavol2.html
12. Khan Z, Pal M, Randhawa H, *et al*. Carriage of Cryptococcus neoformans in the crops of pigeons. *J Med Microbiol.* 1978; 11(2):215-218.
13. Abraham M, Matthews M, John T. Environmental isolation of Cryptococcus neoformans var. neoforrmans from Vellore. *Ind J Med Res*. 1997;106: 458-459.
14. Xavier TF, Auxilia A, Kannan M, *et al*. Isolation and identification of Cryptococcus neoformans from pigeon droppings in Tiruchirappalli district of Tamil Nadu, South India. *Int J Curr Microbiol Appl Sci*. 2013;2(11):404-409.
15. Rosario I, Hermoso de Mendoza M, Deniz S, *et al*. Isolation of Cryptococcus species including C. neoformans from cloaca of pigeons. *Mycoses*. 2005;48(6):421-424.
16. Rosario L, Soro G, Deniz S, *et al*. Presence of C. albidus, C. laurentii and C. uniguttulatus in crop and droppings of pigeon lofts (Columbia livia). *Mycopathologia*. 2010;169(4):315-319.
17. Teodoro VL, Gullo FP, Sardi Jde C, Torres EM, Fusco-Almeida AM, Mendes-Giannini MJ. Environmental isolation, biochemical identification, and antifungal drug susceptibility of Cryptococcus species. *Rev Soc Bras Med Trop*. 2013;46(6):759-764.
18. Nweze EI, Kechia FA, Dibua UE, *et al*. Isolation of Cryptococcus neoformans from environmental samples collected in Southeastern Nigeria. *Rev Inst Med Trop Sao Paulo.* 2015; 57(4):295-298.
19. Gupta KL. Fungal infections and the kidney. *Ind J Nephrol*. 2001; (11):147-154.
20. Dharwadkar A, Vimal S, Buch AC, *et al*. HIV infection presenting as bone marrow cryptococcosis. *Adv Biomed Res.* 2014;3:144.
21. Gugnani H, Gupta N, Shrivastav J. Prevalence of Cryptococcus neoformansin Delhi Zoological Park and its recovery from the sputum of an employee. *Indian J Med Res.* 1972;60:182-185.
22. Yamamoto Y, Kohno S, Noda T, *et al*. Isolation of Cryptococcus neoformans from environments (pigeon excreta) in Nagasaki. *J Jap Assoc Infectious Dis.* 1995;69(6):642-645.

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