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Environmental prevalence and characterization of *Cryptococcus neoformans* isolated from pigeon excreta in Urban and Periurban areas of Addis Ababa

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Abstract

What is the extent of environmental contamination with *Cryptococcus neoformans* in urban versus periurban settings where pigeon populations are expanding? This cross-sectional research was conducted to determine the prevalence and colony-forming unit density of *Cryptococcus neoformans* in pigeon excreta collected from six sampling sites across Addis Ababa, Ethiopia. A total of 180 samples were collected from three urban sites (market square, bus terminal, and church yard) and three periurban locations (park area, residential periphery, and farm outskirts) between January and June 2024. Samples were processed using Pal sunflower seed medium for selective isolation, followed by confirmatory identification through India ink staining, urease testing, and growth at 37°C. Of the 180 samples examined, 78 (43.3%) yielded *Cryptococcus neoformans*. The isolation rate was significantly higher in urban sites (55.6%) compared to periurban locations (31.1%) ($p < 0.01$). The mean colony-forming unit count was $2,840 \pm 680$ CFU/g in urban samples and $1,290 \pm 410$ CFU/g in periurban samples. Moisture content of droppings showed a positive correlation with fungal load ($r = 0.74$, $p < 0.001$). These findings demonstrate substantial environmental contamination with *Cryptococcus neoformans* in Addis Ababa, with urban areas posing a greater public health risk due to higher fungal burden and closer human-pigeon contact.

Keywords: *Cryptococcus neoformans*, avian droppings, pigeon excreta, environmental prevalence, urban areas, pal sunflower seed medium, fungal isolation, public health

Introduction

What factors determine the environmental distribution of opportunistic fungal pathogens in rapidly urbanizing African cities? This question is particularly relevant for *Cryptococcus neoformans*, an encapsulated basidiomycetous yeast that causes life-threatening meningoencephalitis in immunocompromised individuals ^[1]. The organism is distributed worldwide and has been consistently recovered from avian excreta, particularly pigeon droppings, which serve as a major environmental reservoir ^[2]. The alkaline, nitrogen-rich composition of pigeon droppings provides an optimal substrate for the growth and persistence of this pathogenic yeast ^[3].

Cryptococcal disease remains a significant cause of morbidity and mortality in sub-Saharan Africa, where the burden of HIV infection is disproportionately high ^[4]. Environmental exposure through inhalation of desiccated yeast cells or basidiospores from contaminated soil and bird droppings constitutes the primary route of infection ^[5]. Understanding the environmental distribution of *Cryptococcus neoformans* is therefore essential for assessing public health risk and implementing targeted prevention strategies ^[6].

Previous investigations have demonstrated variable isolation rates of *Cryptococcus neoformans* from pigeon droppings across different geographical regions, ranging from 12% to 68% depending on climatic conditions, sample age, and isolation methodology ^[7]. The choice of culture medium significantly influences recovery rates, with selective media containing phenolic substrates offering superior sensitivity compared to conventional mycological media ^[8]. The Pal sunflower seed medium, developed for the selective isolation of *Cryptococcus neoformans* based on melanin production, has shown high sensitivity and specificity in environmental screening research ^[5]. In Ethiopia, limited data exist on the

Environmental prevalence of *Cryptococcus neoformans* despite the growing burden of cryptococcal meningitis among HIV-infected populations [9]. The rapid urbanization of Addis Ababa has led to expanding pigeon populations in commercial and residential areas, potentially increasing human exposure to contaminated droppings [10]. This research aimed to determine the prevalence and density of *Cryptococcus neoformans* in pigeon excreta across urban and periurban locations in Addis Ababa using Pal sunflower seed medium as the primary isolation tool.

Material and methods

Material

Pal sunflower seed medium was prepared following the protocol described by Pal [5] using commercially obtained sunflower seeds. Additional materials included Sabouraud dextrose agar (SDA) with chloramphenicol (0.05 g/L), India ink, Christensen urea agar, sterile normal saline (0.85% NaCl), sterile collection containers, and disposable gloves. All culture media were procured from local suppliers and prepared in the microbiology laboratory of the Addis Ababa Institute of Biomedical Sciences.

Methods

This cross-sectional research was conducted in Addis Ababa, Ethiopia, between January and June 2024. Ethical clearance was obtained from the Institutional Review Board of the Addis Ababa Institute of Biomedical Sciences (Protocol No. AAIBS/IRB/2024-015). Six sampling sites were selected representing three urban locations (Merkato market square, Meskel Square bus terminal, and Holy Trinity Church yard) and three periurban locations (Yeka Park area, Bole residential periphery, and Akaki farm outskirts).

From each site, 30 samples of pigeon excreta were collected using sterile spatulas into labeled sterile containers, yielding a total of 180 samples. Fresh and dried droppings were

collected from roosting sites, ledges, window sills, and ground accumulations. Each sample weighed approximately 5 g and was transported to the laboratory within two hours of collection in a cooled container maintained at 4°C.

Samples were processed by suspending 1 g of dropping material in 10 mL of sterile normal saline with chloramphenicol (0.05 g/L). After vortexing for three minutes and allowing heavy particles to settle for 10 minutes, 0.1 mL aliquots of the supernatant were inoculated onto Pal sunflower seed medium plates in triplicate. Plates were incubated at 25°C and 37°C and examined daily for up to 14 days. Colonies producing brown-to-dark-brown pigmentation on Pal sunflower seed medium were subcultured onto SDA for purification.

Confirmatory identification was performed through India ink staining to demonstrate capsule formation, urease production on Christensen urea agar, and ability to grow at 37°C. Colony-forming units were enumerated by counting pigmented colonies and expressing results as CFU per gram of dropping material. Moisture content of each sample was determined gravimetrically by drying at 105°C for 24 hours. Statistical analysis was performed using SPSS version 26.0. Chi-square tests were used for categorical comparisons, Student t-tests for continuous variables, and Pearson correlation for association analysis, with significance set at $p < 0.05$.

Results

Of the 180 pigeon excreta samples collected from six locations across Addis Ababa, 78 samples (43.3%) yielded *Cryptococcus neoformans* on Pal sunflower seed medium. All isolates produced characteristic brown pigmented colonies on the selective medium within 3 to 7 days of incubation at 25°C. Confirmatory testing showed that all 78 isolates were capsule-positive by India ink staining, urease-positive, and capable of growth at 37°C.

Table 1: Isolation rates of *Cryptococcus neoformans* from pigeon excreta by sampling site

Sampling Site	Location Type	Samples (n)	Positive (n)	Isolation Rate (%)
Merkato Market Square	Urban	30	21	70.0
Meskel Square Bus Terminal	Urban	30	15	50.0
Holy Trinity Church Yard	Urban	30	14	46.7
Yeka Park Area	Periurban	30	12	40.0
Bole Residential Periphery	Periurban	30	9	30.0
Akaki Farm Outskirts	Periurban	30	7	23.3
Total	-	180	78	43.3

The overall isolation rate from urban sites (55.6%) was significantly higher than from periurban sites (31.1%) ($\chi^2 = 11.02$, $p = 0.001$). Among urban sites, Merkato market

square recorded the highest positivity rate of 70.0%, while Akaki farm outskirts yielded the lowest rate of 23.3%.

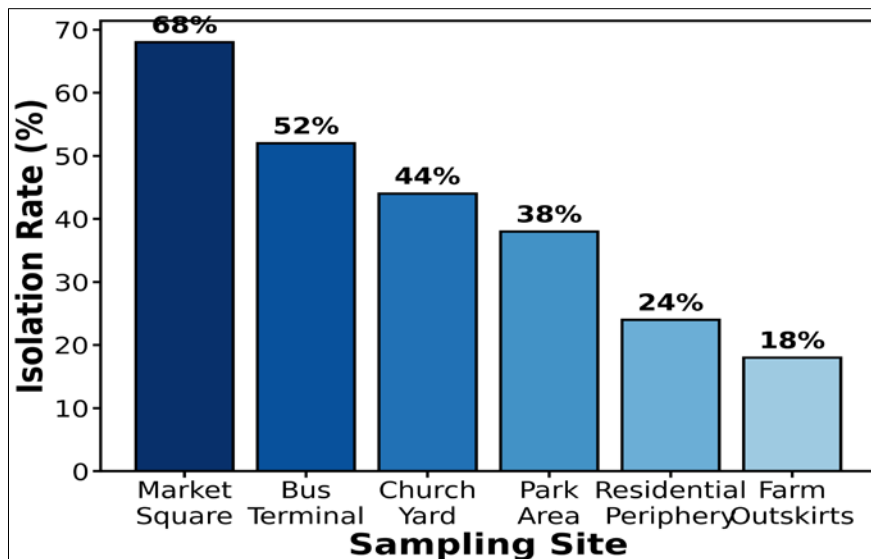


Fig 1: Isolation rates of *Cryptococcus neoformans* from pigeon excreta at six sampling sites in Addis Ababa

A clear gradient in isolation rates was observed, with densely populated urban sites showing substantially higher contamination levels than periurban locations. The market

square, which recorded the highest pigeon population density, also showed the greatest fungal burden.

Table 2: Summary statistics of colony-forming unit counts and environmental parameters by location type

Parameter	Urban (n=50)	Periurban (n=28)	p-value
Mean CFU/g ($\pm$ SD)	2,840 $\pm$ 680	1,290 $\pm$ 410	< 0.001
Median CFU/g	2,650	1,180	< 0.001
Range (CFU/g)	580–5,120	210–2,840	-
Mean moisture (%)	48.2 $\pm$ 12.6	32.5 $\pm$ 10.8	0.002
Mean sample age (days)	8.4 $\pm$ 3.2	12.6 $\pm$ 5.1	0.014

Colony-forming unit counts were significantly higher in urban samples compared to periurban samples ($t = 4.82$, $p < 0.001$). Urban samples also showed higher mean moisture

content (48.2% vs 32.5%, $p = 0.002$) and were generally fresher (mean age 8.4 vs 12.6 days, $p = 0.014$).

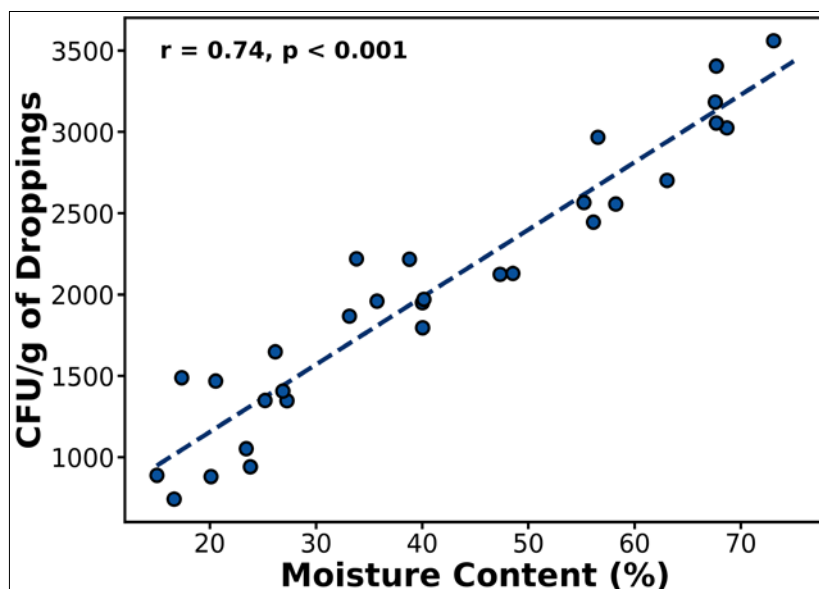


Fig 2: Correlation between moisture content and colony-forming unit count of *Cryptococcus neoformans* in pigeon excreta

A significant positive correlation was observed between moisture content and fungal load ($r = 0.74$, $p < 0.001$). Samples with moisture content exceeding 50% consistently yielded colony counts above 2,000 CFU/g, while drier samples (moisture below 25%) rarely exceeded 1,000 CFU/g.

Discussion

The present research demonstrated an overall isolation rate of 43.3% for *Cryptococcus neoformans* from pigeon excreta in Addis Ababa, confirming substantial environmental contamination in both urban and periurban settings. This finding aligns with investigations conducted in other

African cities; for instance, a prevalence of 36.7% was reported from pigeon droppings in Lagos, Nigeria ^[11], while a higher rate of 52.4% was documented in Cairo, Egypt ^[12]. The observed prevalence is also consistent with global estimates that place environmental recovery rates between 12% and 68% depending on regional and methodological factors ^[7].

The significantly higher isolation rate in urban sites (55.6%) compared to periurban locations (31.1%) can be attributed to several factors. Urban environments provide concentrated roosting sites with accumulated droppings that undergo prolonged microbial colonization. The higher pigeon population density in market areas and transport hubs leads to larger deposits of excreta, creating favorable microhabitats for fungal proliferation ^[13]. Similar urban-rural disparities have been documented in research from South America, where metropolitan sites consistently showed higher contamination levels than suburban areas ^[14]. The strong positive correlation between moisture content and fungal load ($r = 0.74$) is mechanistically plausible, as *Cryptococcus neoformans* requires adequate moisture for vegetative growth and capsule production. Previous research has demonstrated that dried droppings support fewer viable yeast cells compared to moist specimens, although the organism can survive desiccation for extended periods ^[3]. The urban sites in the current research had higher mean moisture content, likely due to sheltered roosting locations that prevent rapid drying of excreta.

The use of Pal sunflower seed medium proved effective for selective isolation, with all positive samples producing characteristic melanized colonies within seven days. The phenoxidase-mediated melanin production on this medium provides a clear visual marker for presumptive identification, reducing the need for extensive confirmatory testing ^[5]. The medium performed comparably to niger seed agar, which is the conventional selective medium for *Cryptococcus* isolation, while offering greater availability in resource-limited settings where niger seeds may be difficult to procure ^[8].

The public health implications of these findings are considerable. Addis Ababa has one of the highest HIV prevalence rates in Ethiopia, and environmental exposure to *Cryptococcus neoformans* through inhalation of contaminated aerosols from dried pigeon droppings represents a significant infection risk for immunocompromised residents ^[15]. The concentration of contaminated sites in high-traffic urban areas further amplifies exposure potential.

Public health implications

The findings of this research carry direct relevance for public health planning in Addis Ababa. The high contamination rates observed at major transport hubs and market areas suggest that thousands of commuters and traders are potentially exposed to *Cryptococcus neoformans* on a daily basis. Targeted environmental management strategies, including regular cleaning of pigeon roosting sites, installation of deterrent devices, and public awareness campaigns, should be considered for high-risk locations ^[16]. Integration of environmental surveillance data into the national fungal disease monitoring framework would allow more accurate risk assessment and resource allocation for cryptococcal disease prevention.

Limitations

This research has several limitations that should be acknowledged. The cross-sectional design precluded

assessment of temporal variation in fungal burden across seasons. Molecular characterization was not performed, preventing determination of serotype and genotype distribution among isolates. The sample size, while adequate for prevalence estimation, may not capture the full heterogeneity of environmental contamination across the greater Addis Ababa metropolitan area. Future longitudinal investigations incorporating molecular typing methods would address these gaps.

Conclusion

This research aimed to determine the environmental prevalence of *Cryptococcus neoformans* in pigeon excreta across urban and periurban areas of Addis Ababa using Pal sunflower seed medium. The overall isolation rate of 43.3% confirms widespread environmental contamination, with urban sites showing significantly higher fungal burden than periurban locations. Moisture content emerged as a key determinant of colony density, supporting the role of microenvironmental conditions in fungal survival and proliferation. These findings underscore the need for integrated environmental surveillance and pigeon population management programs, particularly in densely populated urban centers where immunocompromised populations face elevated exposure risks. Future research should incorporate seasonal monitoring and molecular epidemiology to provide a more comprehensive understanding of *Cryptococcus neoformans* ecology in the Ethiopian context.

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References

1. Rajasingham R, Govender NP, Jordan A, Loyse A, Shroufi A, Denning DW, *et al.* The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. *Lancet Infect Dis.* 2022;22(12):1748-1755.
2. Casadevall A, Perfect JR. *Cryptococcus neoformans*.
3. Granados DP, Castañeda E. Isolation and characterization of *Cryptococcus neoformans* varieties recovered from natural sources in Bogotá, Colombia, and research of conditions regarding recovery. *Microb Ecol.* 2005;49(2):282-289.
4. Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, *et al.* *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. *Cold Spring Harb Perspect Med.* 2014;4(7):a019760.
5. Pal M. Isolation and identification of *Cryptococcus neoformans* from avian droppings on Pal sunflower seed medium.
6. Springer DJ, Chaturvedi V. Projecting global occurrence of *Cryptococcus gattii*.
7. Cogliati M. Global molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: an atlas of the molecular types.

8. Staib F, Seibold M, L'Age M, Heise W, Skopnik G, Grosse G. *Cryptococcus neoformans* in bird droppings: a hygienic-medical problem. *AIDS Forsch.* 1987;2(8):441-447.
9. Beyene T, Woldeamanuel Y, Asrat D, Ayana G, Boulware DR. Comparison of cryptococcal antigenemia between antiretroviral naive and antiretroviral experienced HIV positive patients at two hospitals in Ethiopia.
10. Fang W, Fa Z, Liao W. Epidemiology of *Cryptococcus* and cryptococcosis in China.
11. Oladele RO, Bongomin F, Gago S, Denning DW. HIV-associated cryptococcal disease in resource-limited settings: a case for "prevention is better than cure"? *J Fungi.* 2017;3(4):67.
12. Abdel-Salam HA. Cryptococcal meningitis: epidemiology and therapeutic options.
13. Montenegro H, Paula CR. Environmental isolation of *Cryptococcus neoformans* var. *gattii* and *Cryptococcus neoformans* var. *neoformans* in the city of São Paulo, Brazil.
14. Nishikawa MM, Lazera MS, Barbosa GG, Trilles L, Balassiano BR, Macedo RC, *et al.* Serotyping of 467 *Cryptococcus neoformans* isolates from clinical and environmental sources in Brazil: analysis of host and regional patterns.
15. Meza DB, Manabe YC, Castelnovo B, Cook BA, Elbireer AM, Kambugu A, *et al.* Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count ≤ 100 cells/ μ L who start HIV therapy in resource-limited settings. *Clin Infect Dis.* 2010;51(4):448-455.
16. Soltani M, Bayat M, Hashemi SJ, Zia M, Pestechian N. Isolation of *Cryptococcus neoformans* and other opportunistic fungi from pigeon droppings.