Screening for pathogenic yeasts isolated from pigeon droppings in the Federal District-Pigeons: propagators of pathogenic fungi?

Rastreio de leveduras patogênicas isoladas de fezes de pombos no Distrito Federal-Pombos: propagadores de fungos patogênicos?

Detección de levaduras patógenas aisladas de excrementos de paloma en el Distrito Federal-Palomas: ¿propagadores de hongos patógenos?

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RESUMO

Objetivo: Investigar a presença de fungos patogênicos isolados a partir de amostras de fezes de pombos, em locais de atenção a pacientes imunocomprometidos no DF. **Método:** As amostras foram coletadas em hospitais onde se oferta atendimento a pacientes HIV/AIDS e que tenha uma presença massiva de pombos. Colônias de leveduras foram previamente selecionados em meio Ágar Sabouraud Dextrose acrescido de cloranfenicol, seguindo-se com análise microscópica das estruturas leveduriformes. Colônias de leveduras com suspeita de pertencerem ao gênero *Candida* spp. ou *Cryptococcus* sp., foram inoculadas no meio Ágar Cromogênico para identificação das espécies de *Candida* e em meio Ágar quimicamente definido para indução dos fenótipos de virulência característicos de *Cryptococcus* sp. **Resultados:** 100% das amostras analisadas apresentaram crescimento de leveduras do gênero *Candida* spp. e *Rhodotorula* sp. No meio Ágar Cromogênico foram identificadas nas amostras *C. krusei* em 75%; *C. tropicalis* em 50% e *C. glabrata* em 15%. Em 15% das amostras foi identificado leveduras do gênero *Cryptococcus* sp. **Conclusão:** Dados deste estudo sugerem que fezes de pombo podem estar dispersando leveduras patogênicas e contribuindo com a incidência de infecções fúngicas no DF.

Descritores: Doenças infecciosas; Fezes de Pombo; *Cryptococcus* sp.; *Candida* spp.

ABSTRACT

Objective: To investigate the presence of pathogenic fungi isolated from pigeon stool samples, in places of care for immunocompromised patients in the Federal District. **Method:** The samples were collected in hospitals where care is offered to HIV / AIDS patients and which has a massive presence of pigeons. Yeast colonies were previously selected on Sabouraud Dextrose Agar plus chloramphenicol, followed by microscopic analysis of the yeast structures. Yeast colonies suspected of belonging to the genus *Candida* spp. or *Cryptococcus* sp., were inoculated in the Chromogenic Agar medium to identify *Candida* species and in chemically defined Agar medium to induce the virulence phenotypes characteristic of *Cryptococcus* sp. **Results:** 100% of the analyzed samples showed growth of yeasts of the genus *Candida* spp. and *Rhodotorula* sp. In the chromogenic agar medium, 75% were identified in *C. krusei* samples; *C. tropicalis* in 50% and *C. glabrata* in 15%. In 15% of the samples, yeasts of the genus *Cryptococcus* sp. **Conclusion:** Data from this study suggest that pigeon feces may be dispersing pathogenic yeasts and contributing to the incidence of fungal infections in DF.

Descriptors: Infectious diseases; Pigeon droppings; *Cryptococcus* sp.; *Candida* spp.

RESUMEN

Objetivo: Investigar la presencia de hongos patógenos aislados de muestras de heces de palomas, en los lugares de atención a pacientes inmunodeprimidos del Distrito Federal. Método: Las muestras se recolectaron en hospitales donde se brinda atención a pacientes con VIH / SIDA y que tiene una presencia masiva de palomas. Las colonias de levadura se seleccionaron previamente en Sabouraud Dextrose Agar más cloranfenicol, seguido de un análisis microscópico de las estructuras de la levadura. Las colonias de levaduras sospechosas de pertenecer al género *Candida* spp. o *Cryptococcus* sp., se inocularon en medio de agar cromogénico para identificar especies de *Candida* y en medio de agar químicamente definido para inducir los fenotipos de virulencia característicos de *Cryptococcus* sp. Resultados: el 100% de las muestras analizadas presentó crecimiento de levaduras del género *Candida* spp. y Rhodotorula sp. En el medio agar cromogénico, el 75% se identificó en muestras de *C. krusei*; *C. tropicalis* en 50% y *C. glabrata* en 15%. En el 15% de las muestras, levaduras del género *Cryptococcus* sp. Conclusión: Los datos de este estudio sugieren que las heces de las palomas pueden estar dispersando levaduras patógenas y contribuyendo a la incidencia de infecciones fúngicas en el DF.

Descriptores: Enfermedades infecciosas; Heces de paloma; *Cryptococcus* sp.; *Candida* spp.

Introduction

Systemic mycoses are diseases caused by fungi that infect the body, usually through the respiratory tract, and can spread to other organs.[1] Fungal infections have been growing exponentially in the past few decades, affecting more than a billion people, resulting in approximately 11.5 million fatal infections and more than 1.5 million deaths annually.[1-2] However, despite alarming data, fungal infections are still considered to be neglected.[1]

Although fungi are saprophytes dispersed in the environment, there are reports of the spread of these microorganisms and other pathogens important to public health such as protozoa, bacteria and viruses by pigeons (*Columba livia*).[3-4] Studies suggest that pigeon excreta, which are ubiquitously present in urban areas, may pose a health risk because they allow growth and dispersion of pathogens. This is due to the chemical composition of pigeon feces, rich in nitrogen from uric acid, providing substrate for fungal spores to germinate.[5–7]

Among the microorganisms that compose the pigeon droppings microbiota, it is worth mentioning the presence of yeasts of the genus *Cryptococcus sp*. and *Candida spp*.[10-11] These are opportunistic pathogenic yeasts, responsible for potentially fatal diseases, particularly in immunocompromised patients.

Candida sp. are responsible for 90% of all invasive fungal infections[8], being the fourth leading cause of nosocomial bloodstream infections.[9] Data show that of the isolated Candida species, the predominance is still led by C. albicans (45.5%) followed by C. tropicalis (28.88%), C.krusei (20%), C. glabrata (3.33%) and C. parapsilosis (2.22%).[10-11] In hospitalized patients, *Candida spp*. have been isolated from urine samples (43%), BAL / sputum (18.88%), vaginal smear (8.88%), catheter (7.77%), blood smears and wounds (6.66%), pus (3.33%) and biliary aspirate (2.22%).[9] Although C. albicans is considered by many authors to be part of the human microbiota[12-13], patients with immunity disorders become more susceptible to infection by this microorganism. In addition, the expression of virulence factors associated with a profile of resistance to antifungals may favor colonization and infection by *Candida spp.*

Cryptococcus spp. are encapsulated yeasts frequently found in the environment, particularly associated with pigeon feces [5,14–17.] *Cryptococcus sp*. can invade the central nervous system (CNS) causing fungal meningoencephalitis, which is the most common cause of meningitis in adults living with HIV.[18] Previously, *cryptococcosis* had been attributed to two distinct species, *Cryptococcus neoformans* and *Cryptococcus gattii*. However, the improvement of molecular methods led to a new classification of species, with C. neoformans divided into two species (*C. neoformans and Cryptococcus deneoformans*) and *C. gattii* divided into a total of five species (*C. gattii, Cryptococcus bacillisporus*, *Cryptococcus deuterogattii, Cryptococcus tetragattii and Cryptococcus decagattii*).[19] However, detailed biological comparisons between the various species of *Cryptococcus* have not yet been elucidated, and many studies still consider only the classification *C. neoformans* and *C. gattii*. The species *C. neoformans* is responsible for approximately 220,000 new cases of cryptococcal meningitis annually.[1-2]

Among the aggravating factors of fungal infections already mentioned, it is worth highlighting the scarcity of antifungal treatments, which are becoming

increasingly limited. Currently, only four major classes of antifungals are available to treat life-threatening fungal infections.[20] In addition, the emergence of resistant strains progresses exponentially between different species of fungi.[21] Clinical data report that about 7% of all *Candida* bloodstream isolates are resistant to fluconazole.[22] In addition, different pathogenic fungi are capable of forming biofilms, which are resistant to amphotericin B and azoles, both clinically and in *in vitro* tests, resulting in the use of high doses of antifungals and recurrent treatment failures.[20]

Although the Federal District is a region markedly inhabited by pigeons, present in the most diverse places such as hospitals, restaurants and public transport stations, there are, to date, studies that evaluate the population of fungi present in the pigeon droppings in this region. Thus, the scenario in which pathogenic yeasts could disperse efficiently in environments where care with this type of infection should prevail is very worrying.

If the hypothesis of the dispersion of pathogenic yeasts in a hospital environment is considered, this data may guide the implementation of measures that promote greater patient safety, especially in places of care for immunocompromised individuals. In addition, these data may contribute to the survey of epidemiological surveys in the region, studies that are gaining more and more attention, particularly in the current scenario of the pandemic COVID-19, where the need for these surveys about infectious diseases propagated by animals has become undoubted.

Method

The samples were collected in 2019, at points in the Federal District where care and monitoring is offered to HIV / AIDS patients, another point at the University of Brasília (UnB) and another at the SARAH Hospital, due to the massive presence of pigeons and traffic intense number of individuals. Pigeon feces samples were collected in *pools*, totaling 8 samples. Of these 8 samples collected, 4 samples were collected on the campus of the University of Brasilia-UNB, and 2 samples were collected in the vicinity of the University Restaurant - RU (A 1) and 2 collections at the Central Science Institute - ICC (A2) ; 1 collection at the University Hospital of Brasília - HUB (A3); 1 collection at the Base Hospital of the Federal District (A4), 1 collection SARAH Hospital from Asa Sul - Brasília (A5) and 1 collection from the Regional Hospital of Taguatinga (A6), as shown in Figure 1.

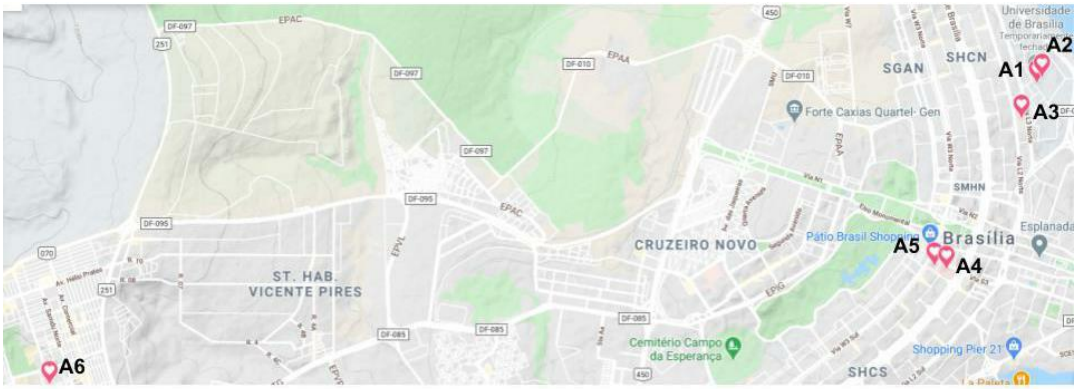

Figure 1- Map indicating the points of sample collection in Brasília. Federal District, 2020.
Legend: The collection sites are signaled with letters ordered from A1-A6 and highlighted in red

balloons.
Source: Google Maps.

The collected samples were placed in sterile plastic tubes and stored in the refrigerator for 24 hours. Then, 0.5 g of each sample was weighed, which were suspended in 5 ml of sterile 0.9% sodium chloride solution. After homogenizing this mixture in vortex for three minutes, the suspension was left to stand for 30 minutes at room temperature, for precipitation of larger structures and contaminants. Then, 100 µl of the supernatant was collected and seeded in Petri dishes containing Sabouraud Dextrose Agar (SD) medium plus the antibiotic chloramphenicol (40 mg / L) (the addition of the antibiotic is to prevent the growth of contaminating bacteria). The plates were then incubated in greenhouses at a temperature of 30 ºC for 48 hours (Figure 2).

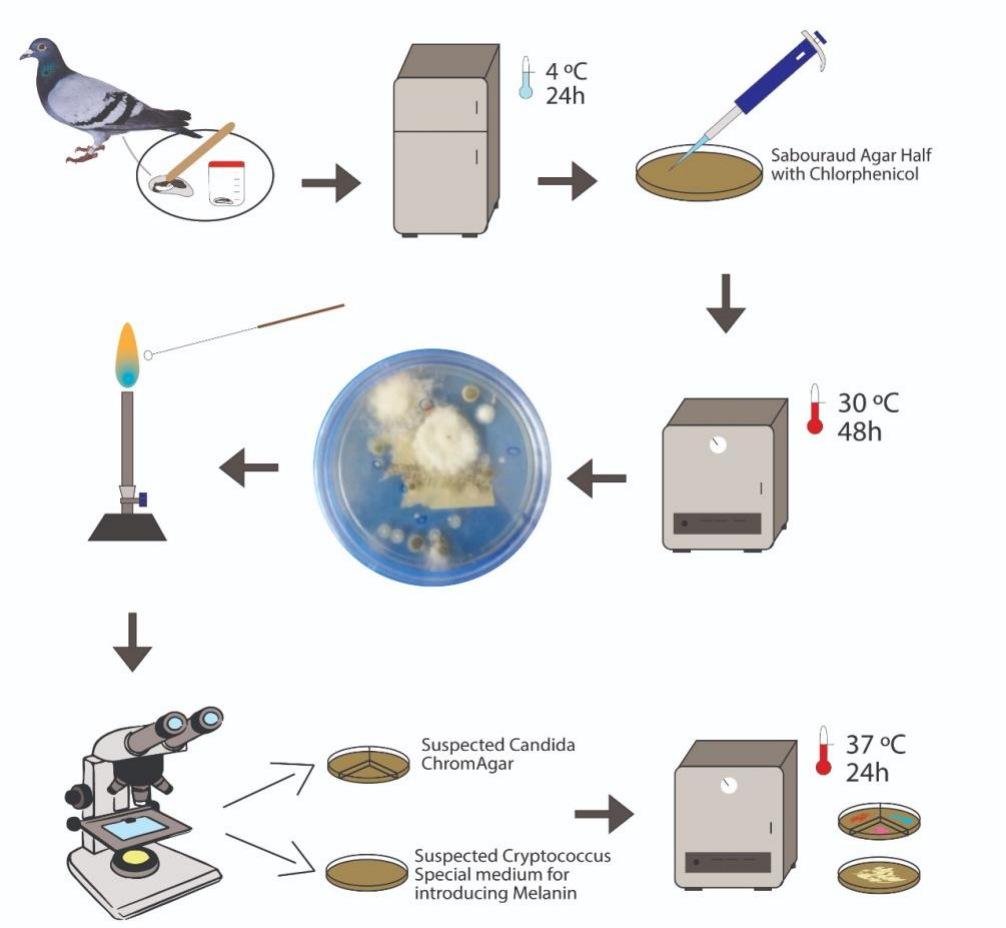

Figure 2- Schematic illustrative of the protocol used in the screening of pathogenic yeasts from a pool of pigeon feces samples, collected in the Federal District. Federal District, 2020.

Analysis and identification of yeasts

Initially, colonies with morphology suggestive of yeast growth in SD + chloramphenicol medium were selected. The selected colonies were stained with methylene blue solution (improves the visualization of fungal cell structures) and observed under optical microscopy in the 40x and 100x ranges. Then, yeast colonies identified under a microscope were inoculated in differential media, which allow genera and fungal species to be identified by biochemical and morphological tests. For qualifying identification of *Candida spp*., the yeasts were

sown on Chromogenic Agar (PLAST LABOR®). To identify the yeasts of *Cryptococcus sp*., The colonies were seeded on Chemically Defined Agar, known as Minimal Medium (MM) (15 mM dextrose, 10 mM MgSO4, 29.4 mM KH2PO4, 13 mM glycine and 3 µM thiamine, pH 5.5, supplemented with 1 mM of L-DOPA (Sigma-Aldrich) that allows the induction of the polysaccharide capsule, specific to the genus, as well as the visualization of the melanin pigment produced by these fungi.[23] After identification of the genera, the yeasts were cryopreserved in aliquots containing BHI medium supplemented with 15% glycerol in a freezer at -20º C, for further analysis and further studies.

Results

Among the samples collected and analyzed, all showed contamination with fungi (Figure 3A). Initially, the samples were sown in SD + Chloramphenicol culture medium, for selection of fungal growth, followed by morphological analysis of yeast colonies. After selecting the yeast colonies, aliquots were collected and stained with methylene blue to view cellular structures in an optical microscope, which allowed the confirmation of yeast structures (Figure 3B-C).

The presence of yeasts from *Candida spp*. was identified in 100% of the samples. The identification of the genera and species of *Candida spp*. it occurred through growth and colorimetric biochemical reaction in Chromogenic Agar medium (Figure 3D). Data analysis demonstrated the presence of *Candida krusei* in 75% of the samples, followed by *Candida tropicalis* in 50% of the samples and *Candida glabrata* in 15% of the samples.

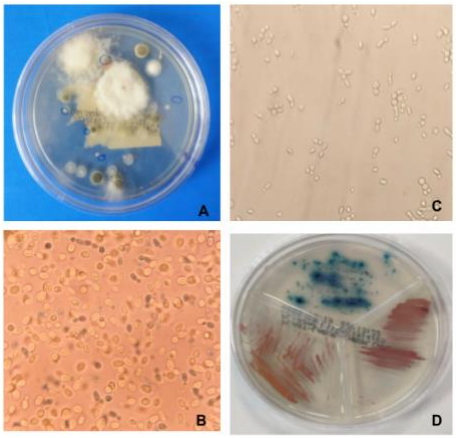

Figure 3 - Identification of pathogenic yeasts in samples of pigeon feces. Distrito Federal, 2020. Caption: A) Sabouraud Dextrose + chloramphenicol medium showing varied growth of fungal colonies. B-C) Optical microscopy images of *Candida* sp. D) Chromogenic Agar Medium with yeast growth of *Candida krusei* and *Candida tropicalis*.

In all samples sown on SD Agar + chloramphenicol medium, the growth of colonies with orange to pink coloration was observed (Figure 4A), and microscopy showed yeast cells (Figure 4B), which according to the Manual of Clinical Microbiology for Infection Control in Health Services of ANVISA, it is a characteristic of yeasts of the genus *Rhodotorula sp.* Such growth was also observed in 100% of the analyzed samples.

In 15% of the samples, the presence of yeasts with bruises was observed, which when grown in MM medium induced the formation of a capsule, visualized with ink ink Nanquim (Figure 4C) and produced the pigment melanin

(Figure 4D), characteristics that correspond to yeasts of the genus *Cryptococcus sp.*

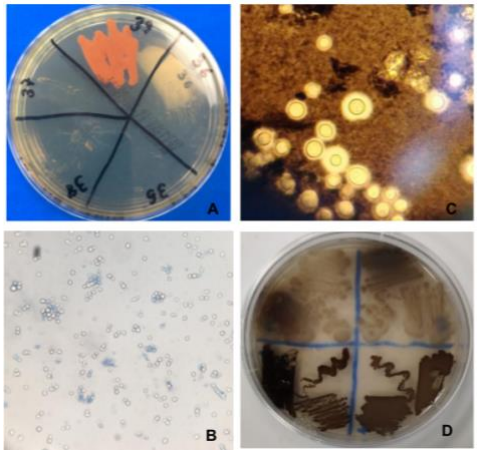

Figure 4 - A) Sabouraud Dextrose Agar + Chloramphenicol Medium *Rhodotorula spp*. B) Optical microscopy images of *Rhodotorula* yeasts. C) Light microscopy images with Nanjing coloring showing encapsulated yeasts of *Cryptococcus spp*. D) Chemically Defined Agar (Minimum Medium) showing colonies with melanin pigment production, typical of *Cryptococcus spp*

Discussion

Pathogenic yeasts are currently an important public health problem, particularly for individuals with some associated immunodepression. Although the health risks associated with fungal pathologies are widely discussed, the topic is considered neglected, even though these infections can cause as many deaths as tuberculosis or malaria.[2,24]

In Brazil, human fungal infections, although known to be prevalent, are not officially notified diseases and this fact makes it difficult to survey precise epidemiological surveys. A study has shown that more than 3.8 million people in Brazil may be suffering from severe fungal infections, mainly patients with malignant cancers, transplant recipients, asthma, previous tuberculosis, HIV infection and those who live in areas endemic for fungi truly pathogenic.[25]

In our study, the growth of *Candida spp*., The etiologic agent of candidiasis, was observed in 100% of the analyzed samples. *Candida* is one of the main yeasts of medical importance, since, according to data provided by the Centers for Disease Control and Prevention (CDC), *Candida* species are in fifth place among pathogens acquired in hospitals and in fourth among bloodstream infections.[5] In addition, these yeasts are developing resistance to antifungal agents for clinical use. Thus, the treatment of candidiasis has been considered a challenge, especially in immunocompromised patients.[24]

According to data from the health department, the Federal District has approximately 12 thousand people infected with HIV / AIDS, who are being assisted by the public and private health system, both receiving drug assistance from the Unified Health System (SUS) as a therapeutic resource.

In the study by Giacomazzi et al., Candidemia rates in hospitalized patients in 2011 in Brazil were 28,991 (total) of which 870 were HIV / AIDS patients, 3,131 were patients with cancer and immunosuppression and 13,336 were patients requiring critical care and surgery.[25] According to the National Cancer Institute[1], the reference hospitals for cancer treatments in the DF, pediatric or not, are the Base Hospitals, SARAH, HUB and the Regional Hospital of Taguatinga, which

were the places chosen for the collection of excrement from the pigeons in this study .

According to data available on the official website of the Government of Brasilia, approximately 45 chemotherapy sessions are performed at Hospital de Base per day.[1] It is worth mentioning that patients undergoing cancer treatment may experience immunosuppression due to both the nature of the disease and the type of treatment.

Among the places of access to hospital care for these individuals, it is worth mentioning the Hospital Universitário de Brasília, where they collected samples of pigeon feces that were contaminated with *Cryptococcus sp*. and *Candida spp*.

One of the most alarming species of the genus *Candida* today is *C. auris*, due to its extraordinary ability to resist different antifungal agents, and corroborating reports that claim that this species is not yet found in Brazil, in our analysis we did not find results suggestive of presence of this species in the samples collected.

Data from the Ministry of Health point to cryptococcosis as the fungal infection with the most frequent hospital interventions in Brazil.[1] Another study reported that the highest rate of cryptococcosis infection was due to an association with AIDS. In Central and South America, 10,548 cases were registered, where the division by countries was carried out and Brazil led the ranking with 53% of the total cases.[26] n our data, yeasts of *Cryptococcus sp*. in 15% of the analyzed samples. The identification of yeasts from *Cryptococcus sp*. occurred through the induction of virulence factors, capsule (essential for survival in phagocytes and an important escape mechanism of the immune system) and melanin (pigment that protects yeasts from oxidative stress).[27] These data suggest that these yeasts are likely to cause disease in animals. However, future tests on animal models may confirm this hypothesis.

Interestingly, in the analysis of pigeon feces carried out in this study, 100% of the samples are contaminated with yeasts of the genus *Rhodotorula spp.* Although *Rhodotorula spp.* be a ubiquitous and saprophytic fungal species, routinely recovered from environmental sources, and for a long time it was considered non-pathogenic, more recent studies suggest this yeast as an opportunistic pathogen, which has the ability to colonize and infect susceptible patients.[28] Reports point out that most cases of *Rhodotorula* infection in humans were fungemia associated with the use of central venous catheters, patients using corticosteroids and immunosuppression.[28]

An alarming point observed in this study was the high population of pigeons on the outskirts and roofs of hospitals, as these places concentrate a greater number of immunocompromised individuals, where health services are provided. Considering that one of the main forms of transmission of some fungal diseases is through the inhalation of viable propagules of fungi, the results of this study indicate the possibility of pigeon droppings playing an important role in the spread of pathogenic fungi in urban areas (Figure 5).

[1] Disponível em: http://www.sgc.goias.gov.br/upload/arquivos/2012-05/proposta_ve-criptococose1.pdf. Acesso em: 11/11/2020.

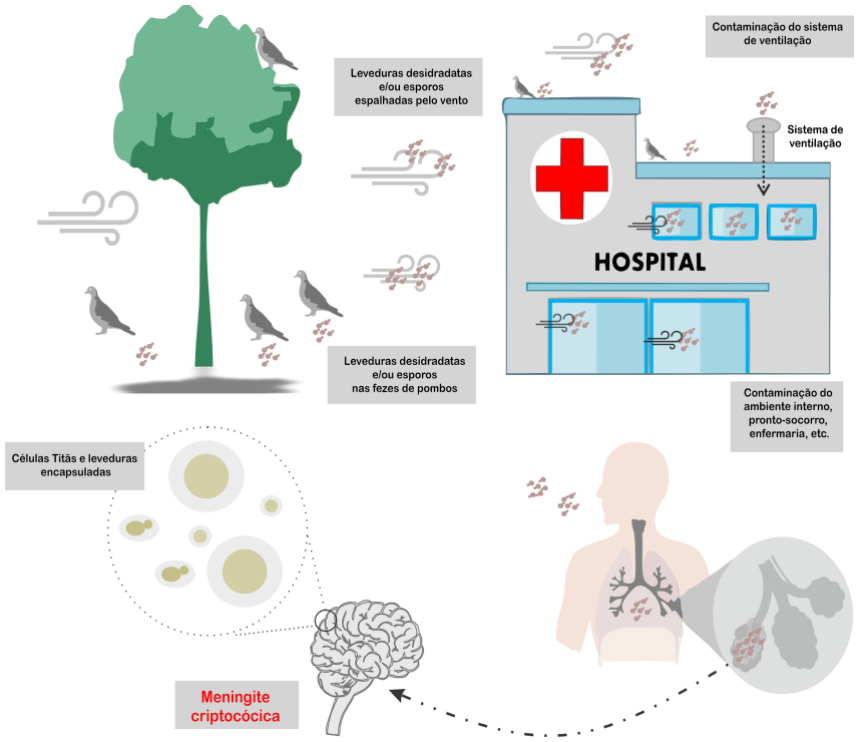

Figure 5- Proposed model for the dissemination of pathogenic yeasts present in pigeon droppings in the Federal District. Federal District, 2020.

In Brazil, especially in Brasília, epidemiological data on fungal infections are rare or nonexistent. Thus, it is evident that these infections are in fact being neglected, requiring preventive measures and information, in the control of the pigeon population and the spread of pathogenic yeasts in the dried feces of these birds, which can be dispersed and spread by the wind, health care environments and urban locations.

It is worth mentioning that, although our data demonstrate the presence of pathogenic yeasts present in the pigeon feces, further studies are needed to ascertain whether these fungal isolates can express virulence factors and develop diseases in an animal model; these analyzes will be carried out in future studies. Still, it is necessary to consider that humans and fungi interact daily, after all, most fungi are saprophytes in the environment, playing a fundamental role in the ecosystem. However, our warning is not for an immunologically healthy population, but for individuals with immunosuppression, who may be more susceptible to these infections, and at the same time, are at greater risk of death.

Conclusion

Due to the time of the COVID-19 pandemic, a disease initially disseminated by interaction between humans and infected animals, studies aimed at investigating the spread of pathogens by animals in the environment have a relevant impact. The initial data in this study are alarming, suggesting that pigeons in the DF may be dispersing pathogenic yeasts and contributing to the incidence of fungal infections.

The samples from the places of care for HIV / AIDS patients proved to be contaminated, emphasizing that these patients have immunosuppression, which makes them more susceptible to fungal infections.

Finally, although there is still a need for further tests, such as investigating the pathogenic potential of these strains isolated from the environment, it is worth highlighting the innovative impact of this study, especially with regard to public health, since it is the first time that this type of screening is carried out in the Federal District, and will certainly be able to contribute to epidemiological surveys of fungal diseases in Brazil, given, as well as fungal infections, neglected. In addition, it is hoped that these data may guide measures of containment in hospital environments, which prevent access of pigeons to ventilation systems in the area, as well as hamper the access of these birds to windows or ventilation doors.

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References

1. Tudela JLR, Denning DW. Recovery from serious fungal infections should be realisable for everyone. Lancet Infect Dis . 2017 Nov;17(11):1111–3. Available from: http://dx.doi.org/10.1016/S1473-3099(17)30319-5
2. Global Action Fund for Fungal Infection Improving outcomes for patients with fungal infections across the world: a roadmap for the next decade . Global Action Fund for Fungal Infection. 2015. Available from: https://gaffi.org/wp-content/uploads/GAFFI_Road_Map_interactive-final0415.pdf
3. Pestka D, Stenzel T, Koncicki A. Occurrence, characteristics and control of pigeon paramyxovirus type 1 in pigeons. Pol J Vet Sci . 2014;17(2):379–84. Available from: http://dx.doi.org/10.2478/pjvs-2014-0056
4. Bodewes R. Novel viruses in birds: Flying through the roof or is a cage needed? Vet J . 2018 Mar;233:55–62. Available from: http://dx.doi.org/10.1016/j.tvjl.2017.12.023
5. Lee WD, Fong JJ, Eimes JA, Lim YW. Diversity and abundance of human-pathogenic fungi associated with pigeon faeces in urban environments. Mol Ecol. 2017;26(17):4574–85. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14216?casa_token=yEyVdKJ7z90AAAAA:hqRqvM_Rr5N9mcPVf8TM9WrTQ84SGKAH9cXTvma0pb4cBYvHV1N-7031B_hdrKyR-GK-uN3dWDnO
6. Pure Air Control Services. Human Health Dangers of Bird Droppings Associated with HVAC Systems . Pure Air Control Services Inc. 2015 [cited 2020 Mar 28]. Available from: https://pureaircontrols.com/human-health-dangers-bird-droppings-associated-building-hvac-systems/
7. Moutinho FFB, Serra CMB, Valente LCM, Borges FVB, de Faria Neto F. Distribuição espaço-temporal das reclamações sobre pombos (columba livia domestica) efetuadas ao centro de controle de zoonoses de niterói, RJ (2009-2013). Hygeia-Revista Brasileira de Geografia Médica e da Saúde . 2015;11(21):49–61. Available from: http://www.seer.ufu.br/index.php/hygeia/article/view/29711
8. Nami S, Mohammadi R, Vakili M, Khezripour K, Mirzaei H, Morovati H.

Fungal vaccines, mechanism of actions and immunology: A comprehensive review . Vol. 109, Biomedicine & Pharmacotherapy. 2019. p. 333–44. Available from: http://dx.doi.org/10.1016/j.biopha.2018.10.075
9. Marak MB, Dhanashree B. Antifungal Susceptibility and Biofilm Production of Candida spp. Isolated from Clinical Samples. Int J Microbiol . 2018 Oct 10;2018:7495218. Available from: http://dx.doi.org/10.1155/2018/7495218
10. Chandra J, Mukherjee PK. Candida Biofilms: Development, Architecture, and Resistance. Microbiol Spectr . 2015 Aug;3(4). Available from: http://dx.doi.org/10.1128/microbiolspec.MB-0020-2015
11. Yano J, Sobel JD, Nyirjesy P, Sobel R, Williams VL, Yu Q, et al. Current patient perspectives of vulvovaginal candidiasis: incidence, symptoms, management and post-treatment outcomes. BMC Womens Health . 2019 Mar 29;19(1):48. Available from: http://dx.doi.org/10.1186/s12905-019-0748-8
12. Ferreira JAG, Carr JH, Starling CEF, de Resende MA, Donlan RM. Biofilm formation and effect of caspofungin on biofilm structure of Candida species bloodstream isolates. Antimicrob Agents Chemother . 2009 Oct;53(10):4377–84. Available from: http://dx.doi.org/10.1128/AAC.00316-09
13. Polke M, Hube B, Jacobsen ID. Chapter Three - Candida Survival Strategies. In: Sariaslani S, Gadd GM, editors. Advances in Applied Microbiology . Academic Press; 2015. p. 139–235. Available from: http://www.sciencedirect.com/science/article/pii/S0065216414000537
14. Nweze EI, Kechia FA, Dibua UE, Eze C, Onoja US. Isolation of Cryptococcus neoformans from environmental samples collected in southeastern nigeria. Rev Inst Med Trop Sao Paulo . 2015 Jul;57(4):295–8. Available from: http://dx.doi.org/10.1590/S0036-46652015000400004
15. de Oliveira Xavier ARE, Cardoso L, Brito RVJ, Nobre SAM, de Almeida AC, de Oliveira AME, et al. Detection and identification of medically important microorganisms isolated from pigeon excreta collected in a university in a newly industrialized country. Biotemas . 2019;32(1):11–20. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=6830107
16. Soltani M, Bayat M, Hashemi SJ, Zia M, Pestechian N. Isolation of Cryptococcus neoformans and other opportunistic fungi from pigeon droppings. J Res Med Sci . 2013 Jan;18(1):56–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23901339
17. Litvintseva AP, Carbone I, Rossouw J, Thakur R, Govender NP, Mitchell TG. Evidence that the human pathogenic fungus Cryptococcus neoformans var. grubii may have evolved in Africa. PLoS One . 2011 May 11;6(5):e19688. Available from: http://dx.doi.org/10.1371/journal.pone.0019688
18. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis . Vol. 17, The Lancet Infectious Diseases. 2017. p. 873–81. Available from: http://dx.doi.org/10.1016/s1473-3099(17)30243-8
19. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol . 2016 Feb;14(2):106–17. Available from: http://dx.doi.org/10.1038/nrmicro.2015.6
20. Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis . 2017 Dec;17(12):e383–92. Available from: http://dx.doi.org/10.1016/S1473-3099(17)30316-X
21. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of

resistance to antifungal drugs challenges human health and food security. Science . 2018 May 18;360(6390):739–42. Available from: http://dx.doi.org/10.1126/science.aap7999
22. Toda M, Williams SR, Berkow EL, Farley MM, Harrison LH, Bonner L, et al. Population-Based Active Surveillance for Culture-Confirmed Candidemia — Four Sites, United States, 2012–2016 . Vol. 68, MMWR. Surveillance Summaries. 2019. p. 1–15. Available from: http://dx.doi.org/10.15585/mmwr.ss6808a1
23. Brandão F, Esher SK, Ost KS, Pianalto K, Nichols CB, Fernandes L, et al. HDAC genes play distinct and redundant roles in Cryptococcus neoformans virulence. Sci Rep. 2018 Mar 26;8(1):5209. Available from: http://dx.doi.org/10.1038/s41598-018-21965-y
24. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med . 2012 Dec 19;4(165):165rv13. Available from: http://dx.doi.org/10.1126/scitranslmed.3004404
25. Giacomazzi J, Baethgen L, Carneiro LC, Millington MA, Denning DW, Colombo AL, et al. The burden of serious human fungal infections in Brazil. Mycoses . 2016 Mar;59(3):145–50. Available from: http://dx.doi.org/10.1111/myc.12427
26. Cogliati M. Global Molecular Epidemiology of Cryptococcus neoformans and Cryptococcus gattii: An Atlas of the Molecular Types. Scientifica . 2013;2013(serotype D):23. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3820360&tool=pmcentrez&rendertype=abstract
27. Brandão FAS, Derengowski LS, Albuquerque P, Nicola AM, Silva-Pereira I, Poças-Fonseca MJ. Histone deacetylases inhibitors effects on Cryptococcus neoformans major virulence phenotypes. Virulence . 2015;6(6):1–13. Available from: http://www.tandfonline.com/doi/full/10.1080/21505594.2015.1038014
28. Wirth F, Goldani LZ. Epidemiology of Rhodotorula: an emerging pathogen. Interdiscip Perspect Infect Dis. 2012; 465717. Available from: http://dx.doi.org/10.1155/2012/465717

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