

Full Length Research Paper

Survey of *Cryptococcus neoformans* in pigeon (*Columba livia*) excreta in Public Square in Umuarama, Paraná, Brazil

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Cryptococcosis is a common opportunistic fungal infection caused by encapsulated yeast of the *Cryptococcus* genus, mainly by the *Cryptococcus neoformans* and *Cryptococcus gattii* species. The fungus is acquired through the inhalation of environmental propagules or, more rarely, through organ transplants in immune-compromised individuals. Domestic pigeons (*Columba livia*) are related to the infection, mainly as natural reservoirs for the fungus, a fact that is relevant due to the large concentration of these animals in public areas, mainly in places of large circulation of people. In order to isolate and diagnose *Cryptococcus* spp., a total of 50 excretion samples from pigeons from five public squares located in the central region of Umuarama, PR, were analyzed, being 10 samples from each square, totaling 50. From the analyzed samples, 100% (50) were negative for the capsulated yeast. Even though all samples were negative, the creation of educative campaigns aiming to raise awareness of the population on the risk of acquiring cryptococcosis and the importance of not feeding the pigeons in leisure areas, the main strategy in the population control of pigeons, were suggested.

Key words: *Cryptococcus neoformans*, yeast, pigeons, zoonosis, public square.

INTRODUCTION

Cryptococcosis is a systemic nature of mycosis caused by *Cryptococcus* fungus genus, whose infection occurs through inhalation of fungus present in the feces of pigeons (Sidrim and Rocha, 2010). Fungi from the *Cryptococcus* genus are composed of 100 species, with two clinically relevant, the *Cryptococcus neoformans* and *Cryptococcus gattii*. This is an sexual encapsulated yeast fungus of the *Filobasidiella neoformans* basidiomycete

(Del Poeta and Casadevall, 2010).

The fungus presents tropism via the central nervous system (CNS), respiratory and integumentary system. The commitment of the immune response is the main predisposing factor for the occurrence of the disease, both in healthy people and immunodepressed patients, bearers of the acquired immunodeficiency syndrome (AIDS) (Pappalardo and Melhem, 2003).

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Mortality by cryptococcosis is estimated at 10% in developed countries, and it can reach 43% in developing countries (Kon, 2008). Within the characteristics observed in *C. neoformans* studies, the presence of capsules is observed. This structure is used in the identification and studies based on antigens that are specific to the mucopolysaccharide capsule. *C. neoformans* has been divided into three varieties and five serotypes, with serotype A belonging to the *grubii* variety (Franzot et al., 1999), serotype D to the *neoformans* variety, and B and C to the *gattii* variety (Sorrell, 2001).

Cryptococcosis is among the emergent fungal infections with significant lethality and morbidity, mainly in the form of meningoencephalitis, which is usually secondary to skin or lung processes, in either immunodepressed or immunocompetent individuals (Lacaz, 2002).

Environments with large concentration of excreta favor the dissemination of the yeast, since the infection by *C. neoformans* is acquired through the inhalation of environmental propagules by dehydrated yeast that can be easily dispersed in the air (Filiú et al., 2002). Due to the high concentration of *C. neoformans* in pigeon excreta, together with the high density of these birds in urban areas and the severity of human cryptococcosis, there is a growing interest in the study of the relationship among the birds, their excreta and the disease in humans (Huffnagle et al., 1999).

From a clinical and epidemiological point of view, cryptococcosis can be opportunistic, cosmopolitan, associated with cellular immune-depression conditions caused by *C. neoformans*. According to Lacaz (2002), cryptococcosis is fundamentally opportunistic, and immunodepression is the relevant factor for the high frequency of this infection.

The objective of this paper was to identify the presence of *C. neoformans* in pigeon excreta collected from squares in the central region in the city of Umuarama, Paraná.

MATERIALS AND METHODS

The city of Umuarama is located in the northwestern region in the state of Paraná, Brazil (latitude 23° 47' 55 south and longitude 53° 18' 48 west) and has 106,387 inhabitants (IBGE, 2012), with population miscegenation of several ethnicities, with different behavioral and cultural habits.

The city has a total of 10 squares, between central and peripheral ones. For the sampling, five squares were selected (Unipar, Artur Thomas, Santos Dumont, Miguel Rossafa and Rodoviaria), as shown in Figure 1, where 10 samples were collected from each square, totaling 50 samples. For the inclusion of the square, the criteria of people flow and large concentration of pigeons were used, these places are located in the central area of the city, with a concentration of shopping streets, which increases the flow of personnel and there is visual observation of the presence of pigeons in these squares.

The collections took place from March to September 2014, and random samples of pigeon feces were chosen in these squares.

With the aid of spatulas and gloves, approximately five grams of old and dry excreta were collected, since they offer substrate that is favorable to the growth of yeast and present lower amount of bacteria, since there is no nutritional competition among these microorganisms (Reolon et al., 2004). The samples were collected on alternate days, between 8 and 9 a.m., in different temperature and humidity conditions, with a few samples being exposed to sun radiation, while others were in the shadows, the minimum temperature was 24.7°C and maximum was 38.7°C.

After collection, the excreta were stored in sterile containers, duly identified and forwarded to the Laboratory of Culture and Microbiology at Universidade Paranaense - UNIPAR, where they were stored at room temperature for 48 h. The samples were homogenized with 2 mL sterile saline solution, the supernatant was aspirated and sown onto Petri dishes; each sample was sown on a plate containing Agar Niger culture medium, as indicated in Santos et al. (2009). The plates were incubated for seven days, at 29 to 30°C (Machado et al., 1993). In order to confirm colonial growth in culture medium with *Cryptococcus* spp., coloration was observed, which ranges from light beige to brown. These colonies were applied to blades microscopic analysis of the fungus and stained with China dye, which is the standard dye for visualization of the fungal capsule, and later analyzed under an optical microscope with 1000x magnification. Colonies with yeast characteristics were sent to urease test, an enzyme that aids the diagnosis (Fisher and Cook, 2001).

RESULTS AND DISCUSSION

Fifty stool samples from pigeons were analyzed, the samples showed no positive results for the isolation and diagnosis of *Cryptococcus* spp. and *Cryptococcus neoformans* is absent in all samples. Environmental sources of *C. neoformans* are very diversified and related to a few organic substrates, among them bird excreta and vegetables. This species is the main etiological agent of cryptococcosis, a disease considered by a few authors as a fatal infection, which can be transmittable to humans in locations with high number of pigeons or by the contact with the corresponding niche through inhalation of a large amount of propagules (Lazera et al., 1993).

The use of the urease test for diagnosing *Cryptococcus* is due to the capacity of the fungus in hydrolyzing urea through a metalloenzyme, resulting in ammonia and carbamate, with a change in the pH of the medium, changing its color from yellow to pink. This ability for hydrolyzing urea is important to differentiate other yeast species, such as *Candida* spp. (Sidrim and Rocha, 2010). The use of the Niger medium provides better visualization and characterization of the colony than the traditional methods for fungal culture. The capacity for producing melanin with the formation of brown colonies in Niger seed extract media is a characteristic of *C. neoformans* and *C. gattii*, not happening in other species from the *Cryptococcus* genus, nor other yeasts of medical interest (Santos et al., 2009).

There are several studies identifying the presence of the *C. neoformans* fungus in bird excreta. However, there are few reports of cryptococcosis in pigeons (Mitchell and

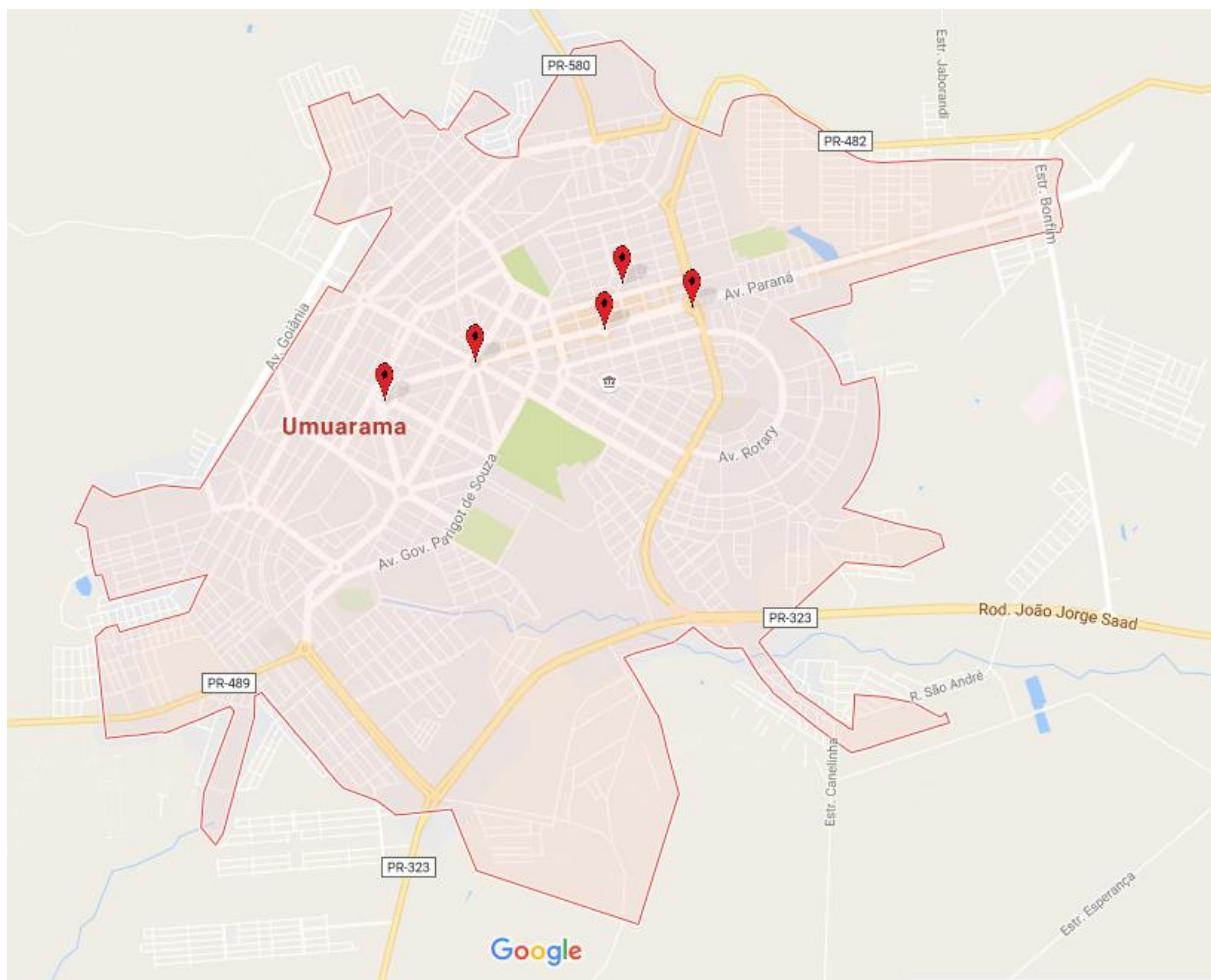


Figure 1. City map of Umuarama, with the location of the five cities (Unipar, Artur Thomas, Santos Dumont, Miguel Rossafa and Rodovaria) where the samples were collected in 2014.

Perfect, 1995), that are resistant to the invasion of the fungus due to their elevated body temperature (Reolon et al., 2004).

According to Bulmer (1990), the problem is the long viability of *C. neoformans* in dry excreta, of approximately two years. Based on this information, old buildings and towers of old churches can be considered potential sources of *C. neoformans* and must be periodically inspected by public health authorities.

C. neoformans is cosmopolitan and opportunistic, especially affecting immunodepressed individuals (Sorrell, 2001). *C. gattii* has a stricter distribution, being usually associated to tropical and subtropical areas (Boekhout et al., 2001). Studies show a variation in the isolation of *C. neoformans* from pigeon excreta ranging from 24.7 to 4.6% (Faria et al., 2010).

Several researchers have found environmental sources of *Cryptococcus* in different regions in Brazil. Silva and Capuano (2008) reported the occurrence of *Cryptococcus*

spp. and parasites of public health interest in pigeon excreta in the city of Ribeirão Preto, São Paulo. In Brazil, clinical and epidemiological studies have shown the importance of cryptococcosis by *C. gattii* for the CNS in young adults of both genders and in children in the North and Northeast regions, with a lethality of 35 to 40% (Correa et al., 1999).

Regarding the isolation of *Cryptococcus* spp. in pigeon excreta, the positivity in our study was low in relation to similar studies performed in Brazil. In the southern region, Reolon et al. (2004) detected the fungus in 100% of the samples in Porto Alegre and Faria et al. (2010) recorded positivity of 26.9% in environmental samples in Pelotas. Abegg et al. (2006) analyzed excreta from 59 captivity bird species kept in cages in a zoo in Rio Grande do Sul, where the birds belonged to 12 different orders, 38 samples found positive for *C. neoformans* in environments with Psittaciformes.

Yamamura et al. (2013) isolated pathogenic fungi from

the *C. neoformans* and *C. gattii* species in the environment represented by the soil where there is a large movement of passers-by in the central region of Londrina, such as parks, squares and streets. It was isolated in eight (2.2%) of 360 samples. These fungi were isolated from the soil with pigeon excreta and vegetable matter near trees in unsheltered areas, and exposed to constant rain and sunlight.

In Lages/SC, Menezes et al. (2014) analyzed 195 samples of pigeon excreta from six squares located in the central region in the city of Lages, SC. From the analyzed samples, 7.69% (15) were positive for the encapsulated yeast. In Cuiabá- MT, Takahara (2013) reported the occurrence of 6.6% *C. neoformans* in 122 samples of dry pigeon excreta collected in 49 locations in the city. Correa et al. (2011) found 100% positive samples for *C. neoformans* in excreta from *Columba* sp. in the city of Cacoal, Rondônia, Brazil.

It is of pivotal importance to guide the population not to feed the pigeons so that there is no proliferation of these animals near homes, schools, squares, churches, sheds, silos and others; as well as closing locations where they are likely to shelter and build their nests (Lima and Lima, 2013).

Cryptococcosis occurs as the first opportunistic manifestation in approximately 4.4% of AIDS cases in Brazil. The prevalence of cryptococcosis associated with AIDS is estimated to be between 8 and 12% in reference centers in the southeastern region (Brasil, 2012). Delgado et al. (2005) and Lindenberg et al. (2008) reported that infection by *C. gattii* in humans and animals has a broader geographical occurrence than what has been habitually described, and its clinical and epidemiological aspect is not well known, therefore, it is necessary to differentiate it from opportunistic cryptococcosis by *C. neoformans*. In regions with large endemism, it is significantly associated with AIDS. However, the authors stated that, on the other hand, *C. neoformans* is capable of causing fatal infection in apparently normal individuals.

Environmental factors might influence n the isolation and diagnosis of the yeast, since the weather in a certain region, allied to temperature, humidity and exposure to sun radiation can influence the recovery and isolation of fungal strains from environmental origin (Quintero et al., 2005).

One of the main strategies is the control of pigeons, and a preventive measure is to damp the locations where there are large accumulation of pigeon feces to avoid the fungus to be dispersed by aerosol. There is no need for notification or isolation of the sick individuals (Hill et al., 1995).

The Ministry of Health does not consider Cryptococcosis as a disease of mandatory notification; therefore, there are no statistical data on its incidence in the city of Umuarama, Paraná. The multiplication of the fungus in

feces and environments with lack of cleaning, together with an increase in temperature, provides the fungus basidiospores, by aerogenous route from the excreta, the contamination of *Passeriformes* and *Psittaciformes* (Lugarini et al., 2008), yeast reservoirs, as well as contamination of other environments near pigeon niche (Rosario et al., 2008).

Conclusion

The low occurrence in the isolation of *C. neoformans* in this study does not exclude the risk for human health, since pigeons are part of the landscape in urban centers, such as in the city of Umuarama, Paraná; there is no need to carry out survey in all the squares of the municipalities in the future.

Conflict of interests

The authors have not declared any conflict of interest.

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