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## Survey of Cryptococcal Antigens in Urban Pigeons (*Columba livia*) in Sao Paulo State, Brazil

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**Abstract:** Cryptococcoses is a zoonosis caused by the saprophyte yeast *Cryptococcus neoformans*. It has been described in several animals species and frequently it is related with environments where bird's droppings are accumulated. The aim of the present study was to investigate the presence of antigens of *C. neoformans* in the blood serum of urban pigeons (*Columba livia*) in São Paulo and Tatui cities, Brazil. During a year 240 birds has their serum evaluated, with a latex agglutination test, for the presence of cryptococcal antigens. All the birds showed negative results. Most researches report the identification of *C. neoformans* in feces present in the environments occupied by pigeons. However, there is little information regarding the investigation of the agent directly on the bird, either by detection of antigen or antibodies. There is no doubt that the fungus remains viable in the dry feces of these birds during years, representing a risk factor for the occurrence of cryptococcoses in susceptible hosts. However, it should be noted that the fungus is often present in the environment due to the presence of substrates favorable to their development. Despite the correlation between the prevalence of *C. neoformans* in nature and bird excreta, the role of the pigeons themselves in disseminating the fungus still need to be more understood.

**Key words:** *Columba livia*, Cryptococcoses, *Cryptococcus neoformans*, Pigeons

### INTRODUCTION

Cryptococcoses is a fungi disease cause by *Cryptococcus neoformans*, an encapsulated saprophytic yeast. This illness is a zoonosis with a worldwide distribution and has been describe in humans and in different animal species (Cherniak and Sundstrom, 1994; Rosario *et al.*, 2008; Velasco, 2000). It is also a major opportunistic mycosis and immunocompromised conditions such as those found in AIDS patients, post-organ transplant surgery or hematological malignancies represent the main factors in the development of this disease (Lindenberg *et al.*, 2008; Leal *et al.*, 2008; Lugarini *et al.*, 2008a; Masur *et al.*, 2002).

Domestic and wild birds can act as possible carriers of fungi that are pathogenic to humans (Carfachia *et al.*, 2006; Costa *et al.*, 2010; Mattsson *et al.*, 1999). Nevertheless it is remarkable the numbers of studies that have been carried out on the occurrence of *C. neoformans* in avian droppings, especially those of domestic pigeons (Faria *et al.*, 2010; Reolon *et al.*, 2004), passerine and psittacine birds (Lugarini *et al.*, 2008b; Raso *et al.*, 2004). Most researches describe the investigation of this fungus in the environmental sources contaminated with bird's feces (Baroni *et al.*, 2006; Carvalho *et al.*, 2007; Faria *et al.*, 2010) however, the serological survey of the cryptococcal capsular polysaccharide antigens in avian species is poorly reported in the veterinary literature (Lugarini *et al.*, 2008b).

Nowadays the use of molecular typing methods allow the comparison between strains from avian to patients, fact that confirmed birds being the source for human infection (Passoni *et al.*, 1998). Even so, despite the recent advances in the molecular biology of *C. neoformans*, aspects of the ecology and epidemiology of this environmental pathogen still remain to be established (Lin and Heitman, 2006). For that reason the proposal of the present report was to investigate cryptococcal antigens circulating in free-living pigeon's sera (*Columba livia*), instead of investigating the presence of *Cryptococcus neoformans* in pigeon's excreta in the environment as already reported in most of the studies performed with pigeons (Faria *et al.*, 2010; Reolon *et al.*, 2004).

### MATERIALS AND METHODS

This study was conducted in urban pigeons (*Columba livia*) that inhabited warehouses used for the storage and marketing of grains and frequented daily by a large number of people as merchants and consumers. With the proper licenses issued by ICMBio/SISBIO (Brazilian Chico Mendes Institute for Biodiversity Conservation/ Biodiversity Authorization and Information System) over one year, every month 10 pigeons of Tatui city and 10 pigeon from São Paulo city were trapped in appropriated cages. All pigeons showed no clinical signs of diseases. Blood samples were collected from the brachial vein of the pigeon with sterile material and

disposed in proper collection tubes. With the use of a commercial kit (Latex-Cryptococcus antigen Detection System Immy<sup>®</sup>, Immuno-Mycologics, INC, Washington, PO BOX 1151, Norman, OK, zip 73071) serum samples were evaluated for the presence of Cryptococcal capsular polysaccharide antigens. According to the manufacturer's protocols 300 µl of serum was added to 50 µl aliquot of pronase, then incubated at 56°C for 30 min. Next, pronase inhibitor was added to the solution. Similar parts of antigen and pronase-treated serum specimen were added onto a ring slide; after five minutes the presence or absence of clots was verified, indicating the antigen-antibody formation. The Latex-Cryptococcus antigen test is based upon the principle that anti-Cryptococcal antibody-coated latex particles will agglutinate with specimens containing cryptococcal capsular polysaccharide antigens. Each test of the present study was performed according to the manufacturer's protocols. Positive and negative controls were tested simultaneously.

## RESULTS AND DISCUSSION

All the 240 pigeon's serum samples (*Columba livia*) evaluated in the present report showed negative results on the Latex-Cryptococcus antigen test. This result is compatible with others studies reported in the Brazilian literature. Lugarini *et al.* (2008a) reports a study where *C. neoformans* polysaccharide antigens were investigated in serum samples from Psittacine and Columbiformes avian species. From 53 pigeon's serum samples collected, only one (1.8%) sample from a wildlife pigeon (*Columba livia*) was positive. Another study published by Costa *et al.* (2010) describes the investigation of *Cryptococcus* sp. in samples from 47 urban pigeon's droppings collected in the environment and in 322 samples collected straight from the cloacae of the birds. *Cryptococcus neoformans* was isolated only from pigeon's droppings collected in the environment. In the other hand Rosario *et al.* (2005, 2009) published studies where *Cryptococcus* sp. were isolated from pigeon's cloacae and crop. From the 331 crop samples analyzed in their first study, 32 (9.66%) samples were positive for *Cryptococcus neoformans*. From the 331 cloacae samples analyzed in their second study, 6 (1.8%) were positive for *C. neoformans*.

Substantial evidence establishes a link between the distribution of *C. neoformans* and pigeons. Nevertheless, whether pigeons are infected or serve as carriers for *C. neoformans* is still debatable (Lin and Heitman, 2006; Nielsen *et al.*, 2007).

The results of some of these studies suggest that regarding avian reservoirs, the main source of *Cryptococcus* sp. might not be the birds themselves, but the environment that they occupy. *Cryptococcus neoformans* lives in surface dirt but in soil it does not compete well with resident microbiota. Nevertheless in

dried avian's droppings the fungus reaches high concentrations and survives for over a year, possibly due to the fact that avian droppings are rich in creatinine, which inhibits other microorganisms (Hirsh and Biberstein, 2004). Probably for that reason the birds' excreta are a favorable environment for the development of *Cryptococcus* species. Also, factors like temperature and humidity influences the proliferation and survival of this fungus. Baroni *et al.* (2006) published a Brazilian study in which the highest rate of isolation of *Cryptococcus* sp. from pigeons dropping samples was recorded in November, pointing out that is spring in Brazil. The serological results obtained in our study were negative in all seasons of the year, even in the spring time where the birds could have potentially more contact with the fungus.

It is important to consider that although the isolation of *C. neoformans* from avian environment may reflect colonization by enrichment due to the favorable conditions of feces-contaminated soil; this does not necessarily mean that birds do not play an active role in the dissemination of *C. neoformans* in nature. The birds could either pass the fungus through their body or carry the fungus on their surface and could readily transport the cells for a long distance (Lin and Heitman, 2006).

According to reports listed in the literature, among birds, pigeon (*Columba livia*) is one of the most important reservoirs for the *Cryptococcus* species (Costa *et al.*, 2010; Haag-Wackernagel and Moch, 2004; Rosario *et al.*, 2009) nevertheless it should be taken into account that the population of free-living pigeons (*Columba livia*) has increased in cities all over the world and undoubtedly the urban flocks are now growing faster than their numbers can be controlled (Reolon *et al.*, 2004). This might be the reason for the long-recognized link between pigeons and fungal diseases in humans. The fact that a high numbers of feral pigeons live in almost every large city in the world (Costa *et al.*, 2010; Haag-Wackernagel and Moch, 2004), leads to a close and frequent contact between human beings and domestic pigeons more than to other avian species. It should be also considered that *C. neoformans* has been isolated from a wide range of avian species besides pigeons, like Passeriformes and Psittaciformes (Lugarini *et al.*, 2008b; Raso *et al.*, 2004; Velasco, 2000). As an example, a report has suggested the transmission of *C. neoformans* from an asymptomatic pet cockatoo to an immunocompromised woman, likely because of direct contact with the bird and its excreta (Nosanchuk *et al.*, 2000).

Cases of human and animal cryptococcoses have increased significantly in recent years. This is mostly due to the improvement in the survival of immunocompromised patients (Haag-Wackernagel and Moch, 2004; Lindenberg *et al.*, 2008). Especially immunocompromised patients need advice about

limiting exposure to *Cryptococcus* sp. potential sources. The literature reiterates that those patients are at considerably higher risk for contracting opportunistic disease. Since opportunistic pathogens have a multitude of vectors besides pigeons, disease prevention is probably best attained by educating the immunocompromised patient to limit contact with all avian sources and to follow guidelines for maintaining hygiene under any circumstance where close contact with pathogenic organisms might occur (Haag-Wackernagel and Moch, 2004; Leal *et al.*, 2008; Mani and Maguire, 2009; Masur *et al.*, 2002).

Moreover, epidemiological screening programs of animal reservoirs are important to identify pathogens that are transmitted to humans. In particular the epidemiological knowledge of cryptococcoses in avian species is important for the understanding of the pathogenesis of infection and the risk factors associated with this illness (Haag-Wackernagel and Moch, 2004; Lugarini *et al.*, 2008a). The diseases aspects of cryptococcal infection are becoming better defined in humans while the life this fungus leads outside the human host remains less well recognized (Lin and Heitman, 2006). Further studies involving, at the same time, birds, their serum, their feces and the environment in which they inhabited, are essential for a better comprehension of the role of avian species in the spread and maintenance of *Cryptococcus neoformans* in the environment. These records are important in the medical literature since prevention of human's exposure to animal-related illnesses requires knowledge of all the different aspects of the zoonosis.

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