

SHORT REPORT

Isolation of *Histoplasma capsulatum* from bats in the urban area of São Paulo State, Brazil

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SUMMARY

The presence of bats in caves, attics, ceilings, and roofs is important epidemiologically as they can increase the chance of human acquisition of pathogens, including *Histoplasma capsulatum*. Brazilian urban areas contain many species of bats, especially insectivorous bats, that are attracted by a wide range of readily available food and shelter. From August 2003 to December 2008, we analysed 2427 bats in the São Paulo State region. Homogenates of the livers and spleens of the bats were plated on specific medium to identify animals infected with *H. capsulatum*. The fungus was isolated from 87 bats (3.6%). The infected bats were identified as *Molossus molossus* (74), *Nyctinomops macrotis* (10), *Tadarida brasiliensis* (1), *Molossus rufus* (1) and *Eumops glaucinus* (1), all insectivorous species. The data presented are a relevant contribution to the epidemiology of *H. capsulatum* in densely populated urban areas such as in São Paulo State, especially since histoplasmosis is not included in the mandatory disease notification system.

Key words: Bats, fungal epidemiology, *Histoplasma capsulatum*, histoplasmosis, *Molossus molossus*, São Paulo urban area.

Histoplasmosis is a systemic mycosis with a worldwide distribution. It is transmitted by inhalation of propagules of the dimorphic fungus *Histoplasma capsulatum*. Soil is the natural habitat of the fungus and human disease is associated with exposure to

infected soil or excreta from birds and bats in endemic areas. The spectrum of disease ranges from asymptomatic acquisition to life-threatening disseminated disease. However, the majority of infections are sub-clinical [1]. Notably, histoplasmosis occurs in immunocompromised [2] and healthy individuals [1]. Clinically significant disease in healthy individuals is often a sequela of exposure to a high inoculum of *H. capsulatum* [1].

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Table 1. Collection location for bats infected with *H. capsulatum* in São Paulo State from August 2003 to December 2008

Location where positive bats were found	Total	%
Roosting in roofs	33	37.9
On the ground	25	28.7
Inside houses	20	23.0
Expansion areas in buildings	4	4.6
Blind box	3	3.4
Hanging from trees	1	1.1
Information not mentioned	1	1.1
Total	87	100.0

There are numerous reports of histoplasmosis outbreaks in individuals exposed to accumulated bat faeces, such as workers involved in cleaning or demolishing buildings [3] or tourists visiting caves containing bats [4]. Histoplasmosis is a relatively common fungal disease in Brazil; however, it is not included in Brazil's mandatory disease notification system and no official data on the disease are available. Our group recently participated in a study evaluating the mortality due to systemic mycoses from 1996 to 2006 in Brazil. Histoplasmosis accounted for 4.3% of deaths, but, when associated with AIDS, the frequency increased to 10.1% [5]. During August 2003 to December 2008, bats were either captured with mist nets by the Zoonosis Control Centre-SP (ZCC-SP) as part of the rabies control programme or were sent by individuals and by other zoonosis control centres from other cities in São Paulo State to our centre for study. Some of these sick or injured bats were caught on the ground. The species recognition of the bats was made according to the identification key of Vizotto & Taddei [6].

The city of São Paulo is the largest economic centre in Brazil and the most populous city of South America, with about 10.9 million inhabitants in an area of 1523 km². The other cities in the state of São Paulo that participated in the study are Guarulhos with 1.2 million inhabitants (area 318 km²); Osasco with 700 000 inhabitants (area 65 km²); Jundiaí with 340 000 inhabitants (area 432 km²); and Mogi Mirim with 84 000 inhabitants (area 499 km²) [7].

Bats captured and sent to ZCC-SP were anaesthetized with ketamine (80 mg/kg), euthanized in a CO₂ chamber and transferred to a laminar flow hood with air released through a HEPA filter (class II bio-safety hood). The spleens and livers were aseptically

removed and macerated in saline with chloramphenicol (200 mg/l). Each organ sample was cultured on brain heart infusion agar with chloramphenicol supplemented with 0.1% L-cysteine and 1% glucose and Sabouraud dextrose agar at 25 °C and 37 °C. The cultures were incubated for 60 days and observed weekly. Bats sent from outside the ZCC-SP were similarly processed immediately after receipt. The Scientific Committee of the Zoonosis Control Center and the ethical committee of the Institute of Biomedical Sciences of the University of São Paulo approved the study.

Presumptive colonies of *H. capsulatum* were plated on Mycosel agar for identification of the isolated fungi. After growth of colonies, slide micro-cultivation was performed and the characteristic structures of the fungi were identified to confirm the diagnosis.

A total of 2427 bats were collected. The majority (1728, 71.2%), were from the city of São Paulo and 699 (28.8%) were from neighbouring cities. *H. capsulatum* was recovered from 87 bats (3.6% of all bats examined). Infected bats were identified from five cities: São Paulo (70 bats, 80.5% of total infected bats), Guarulhos (10 bats, 11.5% of total infected bats), Jundiaí (four bats, 4.6% of total infected bats), Osasco (two bats, 2.3% of total infected bats) and Mogi Mirim (one bat, 1.1% of total infected bats).

The families of bats captured were Molossidae (1391 bats), Phyllostomidae (812 bats), Vespertilionidae (203 bats) and Emballonuridae (one bat). Twenty bats had no family identified. Twenty-three genera and 42 species of bats were identified. Gender determination identified 1126 males and 1279 females, with an additional 22 bats in which gender could not be determined. All 87 isolates of *H. capsulatum* were from bats in the Molossidae family (four genera and five species), which represented the largest group examined (57.3%). Sixty-four (62%) isolates were from *Molossus molossus* (18 males, 56 females), while the other four infected species were *Nyctinomops macrotis* (one male and nine females), *M. rufus* (one male), *Tadarida brasiliensis* (one male) and *Eumops glaucinus* (one male). We also determined bat age by assessing forearm epiphyses, since it is cartilaginous in immature bats and calcified in adults. Eight of the bats were young and 79 were adult.

The gender difference in infected animals is statistically significant (χ^2 test, $P=15.67$, $\alpha=5\%$). The female predominance is presumably related to the longer time spent in the colony in care of the

offspring, with increased time of exposure to *H. capsulatum* spores. In some species like *N. macrotis*, females live in maternity colonies from which males are segregated, which may also contribute to increased contamination of the females. Further study is necessary to validate this hypothesis.

The fact that the Molossidae family represented the largest group of bats examined was expected because this family is the most common in the urban area of São Paulo [8]. *M. molossus* was the most frequent species identified and this species is known to frequently shelter in roofs and ceilings of houses and other spaces within buildings. Therefore, the number of complaints about this bat is greater, resulting in increased capture by the ZCC-SP.

Infected or sick bats were captured in diverse environments. Locations for capture were in roofs, on the ground, inside houses, around the external area of houses, within blind boxes, and hanging from trees. Table 1 details the number of bats captured and the different types of location. Notably, most bats that had fungi in the organs were apparently healthy. In contrast, the bats caught on the ground appeared sick.

The majority of infected bats were captured roosting in roofs in areas of dense human population, consequently increasing the risk of individuals in these areas for histoplasmosis. Importantly, the risk depends on the degree of exposure to contaminated soil or guano and the immunological status of the individual, such that high inoculum exposure and immunodeficiency significantly increase the risk of a life-threatening disease [1, 2]. Several species of bats with systemic histoplasmosis have protracted excretion of viable fungi in their faeces [9]; however, it is possible that there is inter-species variation.

Of the five infected bat species identified in the present work, *H. capsulatum* infection has previously been reported in *T. brasiliensis* and *M. molossus* [10]. This report is the first to identify histoplasmosis in *N. macrotis*, *E. glaucinus* and *M. rufus*. In 2007, histoplasmosis was identified in two *N. macrotis* and one *E. glaucinus* captured in the same shelter. Such findings underscore the risk to public health of urban shelters infected with bats and clearly indicate the urgent need for regular surveillance in these areas. The knowledge of the natural history of histoplasmosis in urban areas is important for the establishment of preventive strategies and disease control.

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DECLARATION OF INTEREST

None.

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