



Histoplasmosis outbreak in Tamboril cave—Minas Gerais state, Brazil



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ABSTRACT

In this study, we report four cases of *Histoplasma capsulatum* infection in eight biologists who made an expedition to determine the prevalence of this fungus in a cave localized in the northwest of Minas Gerais state, Brazil. This case report demonstrates the importance of evaluating the *H. capsulatum* presence in Brazilian caves before opening to public visitations.

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1. Introduction

Histoplasmosis is a cosmopolitan systemic mycosis caused by the fungus *Histoplasma capsulatum*. This disease has been already reported in USA's Middle West and Northeast; Latin America, Asia, Africa and several cases with no etiological confirmation in Europe [1]. Histoplasmosis is the most prevalent mycosis disseminated in Central America, United States (Ohio and Mississippi) and Mexico's southeast [2]. Although histoplasmosis can affect healthy individuals, the disease has been frequently related to immunocompromised patients [1]. Severe manifestations are common with rapid progression being in many cases lethal. Facing that, a precise and quick diagnosis is needed to ensure efficient treatment [3].

The dimorphic fungus *H. capsulatum* lives as a saprophytic form in soil, especially in alkaline conditions (pH 5 to 10) and temperatures between 20 to 30 degrees. The infection occurs after initial exposure to *H. capsulatum* by inhaling the spores present in the environment. Once inhaled into the alveoli, the organism readily spreads in macrophages throughout the reticuloendothelial system [4,5]. At the same time, the conidia transform into yeast-like

cells, characterizing the dimorphism of this species. Studies in vitro revealed that conidia may transform into yeast-like cells efficiently whether or not they are phagocytosed by macrophages cells, but not when ingested by dendritic cells, suggesting that these cells restrict transformation into the yeast stage [6].

H. capsulatum can survive at extreme conditions and change its microenvironment to achieve pathogenicity [7]. Many foci were observed after accidental exposure to great amount of bird and bat feces on endemic and non-endemic areas [8]. There are some case reports describing infections caused by *H. capsulatum* after tourism trips [9]. In Central America, a histoplasmosis burden in a tourist group that visited caves was reported. It confirms the importance of the fungus identification in caves before opening for tourist visitors and workers [10].

The present work discusses a case of four immunocompetent researchers that developed different clinical aspects of histoplasmosis after bat's guano collected in a private cavern named Tamboril, located in the Minas Gerais state, Brazil. The clinical report varied from pulmonary to systemic commitments that were evidenced by X-ray and clinical investigation.

2. Case

The cave of Tamboril has 2.5 km extension with a diverse calcite formation and habitats such as a lake. It is located at 150 km from Brasilia, Brazil capital. Its fauna is composed mostly

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by different populations of bats and insects that live on bat guano. This cave was open to visitation until the land was bought by a calcium miner enterprise.

After several years closed and in order to reopen the cave to visitors, the enterprise employed a group of eight biologists and cavers investigate *H. capsulatum* occurrence as well as other fungal species. Facing that, bat's guanoses were collected at different cave locations.

The researchers used whole attire and adopted measures appropriated for this study. However, due to the intense heat and long collection periods (almost 11 h) inside the cave, the researchers took off their masks several times, enabling a possible inhalation of fungal spores. Subsequently, in the 10th day after the guano exposure, four of the eight researches presented with histoplasmosis symptoms.

The researchers, with ages varying from 20 to 35 years old, were assisted by infectiologists of Federal University of Minas Gerais's medicine Clinical Hospital, Belo Horizonte, Brazil. Four of the eight researchers who had entered the cave presented with clinical symptoms including lethargy and night fever of body temperature ranging from 37.5 to 38.5 °C. No cervical or generalized lymphadenomegaly as well as other clinical manifestations were observed at this stage. Complete blood counting (CBC) was performed on the day 12 showing normal hemoglobin, leucocytes levels and erythrocyte sedimentation rate. On the other hand, C-reactive protein dosage was higher considering reference levels in three of the four researches (Table 1).

The serological tests using the anti-*H. capsulatum* antibody were done on day 15, for the four patients, and were all negative. Chest radiography demonstrated nodular diffuse shadowing and calcifications as described in pulmonary infections such as histoplasmosis (Fig. 1).

The mycological tests were made by sputum analysis and culture for the four patients. Fresh sputum was examined under the microscope after 10% potassium hydroxide (KOH) treatment, followed by Lactophenol cotton blue staining. It was observed numerous small (7 µm) and oval yeasts, characteristic of the specie *H. capsulatum* (Fig. 2A). For fungi isolation, the sputum was disseminated on Agar-Sabouraud plaques containing Chloramphenicol 0.05%. After two weeks, beige creamy colonies (yeast form) as well as light beige, cotton colonies (filamentous) presenting *H. capsulatum* macromorphological characteristics, were observed. Microscopic analyses were made after staining colonies fragments with Lactophenol cotton blue dye and showed numerous characteristics of *H. capsulatum* including alkaline septate hyphae, brown macroconidia with thick and cog wall, 1-celled, spherical, 8–14 µm diam., (Fig. 2B).

Two of the four researchers numbered as 1 and 3 developed severe symptoms as arthritis and arthralgia in the knees and

elbows; swollen hands and legs; permanent fatigue and respiratory symptoms prolongation. Therefore, antifungal treatment was initiated on day 20, with oral itraconazole 400 mg per day for 14 days. After one week of treatment (day 27) these symptoms disappeared. The cure after this therapy strongly suggests a fungal infection as a diagnostic treatment proof. The other two researchers (2 and 4) showed spontaneous cure without antifungal therapy with no disease symptoms on the day 27.

3. Discussion

Histoplasmosis is endemic on tropical and temperate zones over the world, which have temperatures between 20 and 30 degrees and high humidity. This disease is endemic in Mississippi, Ohio, Caribbean, south of Mexico, South America, Africa, Asia, and in some areas of Central Europe. On these regions, *H. capsulatum* is frequently found in soil contaminated with birds and bats feces [8,11–13]. In an undesirable manner, our results demonstrated that the *H. capsulatum* was present on the explored environment. The way that the infection occurred made clear that pathogen identification is crucial to understand and diagnose the fungus infection. The specific and rapid diagnosis is important mostly because the clinical symptoms can be confused with other pathologies such as pneumonia and tuberculosis [11].

Although the researches dressed individual protective equipment during guano collection, the infections strongly suggest that the cave was infested of *H. capsulatum* spores. It also demonstrates that disease progression is related to individual immune system competence. Facing that, expeditions into bat-colonized caves can result in different infections frame according to the host immune system and previous pathogen contact.

The four patients who developed symptoms had never visited the cavern whereas the other four researches that had no symptoms visited the cavern previously. This fact suggests that these individuals might be resistant to the infection as they probably had previous contact or even subclinical infection. When the disease is readily diagnosed the treatment reduces the infections symptoms within 3 days [14].

Histoplasmosis can progressively disseminate and is diagnosed by chest radiograph, histology and microbiology and if it is not treated, it can be fatal [1,15,16]. Facing that, the health programs should develop to investigate fungus incidence, mostly on tropical areas as observed in Brazil [2,17]. Understating the possible fungus reservoir and the description of its endemic area, we can prevent disease transmission avoiding the infection [18].

Another way to control fungi infestations is to clean the environment with fumigation with Formaldehyde 3.8% and take off the guano. However, this practice has a high risk to environment once

Table 1
General characteristics of the researchers.

Researcher	Age years	Gender	Type of infection	Night fever	WBC [$10^3/\mu\text{L}$]	Serology	Protein C -reactive	X-ray Fig. 1	Treatment
1	25	Female	Acute	Yes	5.40	Negative	9.8 mg/L	1 A and B	Yes
2	20	Male	Acute	Yes	7.56	Negative	6.2 mg/L	1 C and D	No
3	33	Male	Acute	Yes ^a	5.93	Negative	61.1 mg/L	1 E and F	Yes
4	29	Female	Acute	Yes	8.96	Negative	48.5 mg/L	1 G and H	No
5	35	Male	Asymptomatic	No	nd	nd	nd	nd	No
6	23	Male	Asymptomatic	No	nd	nd	nd	nd	No
7	24	Male	Asymptomatic	No	nd	nd	nd	nd	No
8	26	Male	Asymptomatic	No	nd	nd	nd	nd	No

nd: Not determined.

Treatment Posology—Itraconazole, 400 mg daily for 14 days.

Serology available in Brazilian laboratories is the double radial immunodiffusion.

WBC—White Blood Cell count in microliter of blood in the day 12.

^a The research 3 presented fever daytime, with worsening between the 15th and 20th day.

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