Title

Histoplasma capsulatum in Cayenne, French Guiana

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Abstract

We carried out a soil sampling survey in September 2008 in central Cayenne, French Guiana, using molecular methods to assess the presence of the dimorphic fungus *Histoplasma capsulatum*. Four of the 31 samples collected (12.9%) tested positive by PCR, with confirmation of the result by DNA sequencing. *Histoplasma capsulatum* is therefore present in urban environments in French Guiana. These results provide additional support for the primary prophylaxis of AIDS-related histoplasmosis in French Guiana.

Introduction

Histoplasmosis is a systemic mycosis affecting humans and animals. It is caused by inhalation of the spores of the mold form of the dimorphic fungus *Histoplasma capsulatum* from contaminated environments. It may also result from the reactivation of a latent infection in a context of immunodeficiency. Previously regarded as a rare disease, it has emerged since the late 1980s as a major opportunistic infection in people with AIDS living in endemic areas, such as the Americas [1]. In French Guiana, histoplasmosis is now the opportunistic infection most frequently associated with HIV/AIDS, together with tuberculosis, and is the leading cause of AIDS-related death. About 65% of patients suffering from AIDS-related histoplasmosis in this region are immigrants from other countries in which this disease is known to be endemic (Brazil, Suriname, Guyana, Haiti), and in most cases, the origin of the infection remains unknown [2]. Since the first isolation of this fungus from soil in 1949 [3], most ecological studies have associated the presence of *Histoplasma capsulatum* in the environment with bird and bat droppings [4]. In French Guiana, *Histoplasma capsulatum* was isolated in 1955 from environmental samples collected from a chicken farm implicated in a family outbreak of histoplasmosis [5]. No other ecological studies have since been performed. We report here the detection, by molecular methods, of *Histoplasma capsulatum* in soil samples collected from central Cayenne in September 2008.

Materials and Methods

We collected 31 independent soil samples from central Cayenne (Fig.1). The samples were collected in sterile flasks. They were obtained from superficial layers of the ground from the dark narrow spaces between houses and buildings (n=26) and from the bases of trees (n=5). Sampling sites were selected on the basis of the likely presence of guano or bats. DNA was extracted from 150 mg of each soil sample with the PowerSoil® DNA kit (MO BIO, Carlsbad, CA), according to manufacturer's instructions, after three cycles of freezing at -

80°C for 10 min and boiling for 10 min to disrupt fungal cell walls. The extracts obtained were screened for the presence of *Histoplasma capsulatum* with the real-time PCR method developed at the Parasitology-Mycology Laboratory of Cayenne Hospital for use in medical diagnosis [6]. For confirmation of the presence of the fungus in samples testing positive in this initial screening, we designed a nested PCR targeting a specific sequence in the internal transcribed spacer region of the rRNA gene complex of *Histoplasma capsulatum* (GenBank accession number: AB055231). The outer PCR was performed with the primers F15 (5′ CTG GGA GCC TCT GAC CG 3′) and R490 (5′ CAG AGC GGG TGG CAA AG 3′) and amplified a 476 bp fragment. The inner PCR was performed to ensure specificity, with primers F59 (5′ CCC TTG TCT ACC GGA CCT GTT 3′) and R438 (5′ ACT GCA TTT CGG GCA CGT 3′) and amplified a 380 bp DNA fragment (Fig.2). The first PCR amplification was performed in a 50 µl reaction mixture containing 5 µl of the template DNA suspension extracted from the samples, 0.2 mM of each deoxynucleotide, 20 pmol of each primer (primers F15 and R490) and 1 U Phusion® DNA polymerase (Finnzymes, Espoo, Finland) in 1× PCR buffer. The reaction conditions were as follows: 98°C for 10 s, followed by 35 cycles of 98°C for 5 s, 57°C for 10 s and 72°C for 45 s, with a final extension at 72°C for 1 min. We then transferred 1 µl of the PCR mixture to a new tube containing 49 µl of reaction mixture and the F59 and R438 primers. The conditions of the second PCR were as follows: 98°C for 10 s, followed by 30 cycles of 98°C for 5 s, 58°C for 10 s and 72°C for 45 s, with a final extension at 72°C for 1 min. The products of the second PCR were then characterized by direct DNA sequencing and compared with the sequences in the GenBank nucleotide database.

Results

Four of the 31 samples (12.9%) tested positive for *Histoplasma capsulatum* by real-time PCR (Threshold cycle value (Ct) was 22.78 for sample 1 (S1), 35.92 for S2, 33.73 for S3, 26.10 for

S4). These results were confirmed by DNA sequencing (GenBank accession numbers: JF826013 to JF826016). Of note, attempts to isolate *Histoplasma capsulatum* by a direct culture method [7] were successful for only one of the samples testing positive by PCR. All the other plates were rapidly overgrown by contaminants.

Discussion

These results demonstrate that *Histoplasma capsulatum* is present in urban environments in French Guiana. Bats commonly roosted over the soil sampling sites, suggesting a possible link between these mammals and the presence of *Histoplasma capsulatum* in central Cayenne. Fifteen bat species are recorded as present in the city of Cayenne [8]. They mostly inhabit shaded areas, such as roofs, attics and walls, occupying small openings and crevices during the day, as observed during sample collection. The predominant bat species found in urban environments in French Guiana, *Molossus molossus*, has already been shown to harbor *Histoplasma capsulatum* [9]. This fungus was also recovered in culture, from liver and spleen samples from a dead specimen found in a house in Cayenne [Christine Aznar, unpublished data]. Symptomatic histoplasmosis is not particularly prevalent in immunocompetent patients in French Guiana, although it is almost certainly underdiagnosed (eight biologically confirmed cases at Cayenne Hospital over a period of 10 years) [unpublished data], so levels of exposure to *Histoplasma capsulatum* spores may be limited. However, spores may be present in sufficient numbers to act as a potential source of contamination for AIDS-related histoplasmosis, which occurs, in French Guiana, in patients with a highly compromised immunity status (80% of them with CD4 cell count < 100/µl [2]), frequently in those with additional risk factors, such as poor living conditions or crack cocaine use [10]. Recent epidemiological studies of times series of first episodes of disseminated histoplasmosis in French Guiana have suggested that a large proportion of cases result from recent exposure

[11]. These results provide support for the primary prophylaxis of AIDS-related histoplasmosis in French Guiana.

Acknowledgments

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Figures

Fig.1 Map of sampling sites in central Cayenne (● positive samples, ○ negative samples)

Fig.2 Nested PCR gel (M: Marker, N: Negative control, P: Positive control)

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