

Cryptococcus neoformans and *Histoplasma capsulatum* in dove's (*Columbia livia*) excreta in Bolívar State, Venezuela

Julman R. Cermeño,* Isabel Hernández,* Ismery Cabello,** Yida Orellán,* Julmery J. Cermeño,** Rosa Albornoz,* Elba Padrón,* Gerardo Godoy*

ABSTRACT. Dove's excreta samples from state Bolívar several places in Venezuela, were evaluated to determine the presence of primary pathogen fungi in dove's excreta. Filamentous fungi such as: *Aspergillus* spp (31.1%), *Mucor* spp (20.2%), *Penicillium* spp (9.5%) and *Fusarium* spp (6.7%) were the most frequently isolated strains. Species such as *Candida albicans* (4.1%), *Cryptococcus albidus* and *Rhodotorula* spp (2.7%), *C. neoformans* var *neoformans* (1.4%), *Trichosporum asahii* (1.4%), *Curvularia*, *Microsporum* and *Phoma* as well as *Histoplasma capsulatum* (1.3%) were less frequently isolated. This study shows the presence of *C. neoformans* and *H. capsulatum* in dove's excreta from Bolívar state, it remarks infection risk with these pathogens fungi and the necessity to avoid accumulation of dove's excreta.

Key words: Bird's droppings, environmental isolates, pathogens fungi.

RESUMEN. Se estudiaron muestras de excretas de palomas obtenidas de varios lugares del estado Bolívar, Venezuela, con la finalidad de determinar la presencia de hongos patógenos primarios. Hongos filamentosos tales como: *Aspergillus* spp (31.1%), *Mucor* spp (20.2%), *Penicillium* spp (9.5%) y *Fusarium* spp (6.7%) fueron los aislados más frecuentes. Especies como *Candida albicans* (4.1%), *Cryptococcus albidus* y *Rhodotorula* spp (2.7%), *C. neoformans* var *neoformans* (1.4%), *Trichosporum asahii* (1.4%), *Curvularia*, *Microsporum* y *Phoma*, así como de *Histoplasma capsulatum* (1.3%) se aislaron con menor frecuencia. Este estudio demostró la presencia de *C. neoformans* y *H. capsulatum* en excretas de palomas del estado Bolívar, lo cual implica un riesgo de infección con estos hongos patógenos y la necesidad de evitar la acumulación de estas excretas.

Palabras clave: Aislados ambientales, excretas de aves, hongos patógenos.

INTRODUCTION

Several epidemiological studies have shown the distribution of pathogenous fungi like *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* and *Aspergillus* spp, related to apparition of animal and human infections in these areas.^{5,38} *C. neoformans* has been isolated from a great variety of sources like fruits, vegetables, wood, soil contaminated with bird's excreta, wasp's nests, flowers, bark of trees, particularly of *Eucalyptus* (*E. camaldulensis*, *E. tereticornis*, *E. citriodora*, *E. rudis*) and bird's excreta. In regard to the later, dove's excreta is considered the most important source for human infection.^{15,33,36} The epidemiological relationship between *Cryptococcus* habitat and human infection is cir-

cumstantial since the global distribution of *C. neoformans* var *gattii* infection has been associated to that of *Eucalyptus* trees.^{28,36} *H. capsulatum* is a geophyllus fungus that grows naturally in a multicellular filamentous form³⁸. This fungus has been found all over the world. *H. capsulatum* var *capsulatum* is the only species described in the American continent, while *H. capsulatum* var *duboisii* is endemic in Central and West Africa and Madagascar.^{27,39} In Venezuela, it has been isolated from soil in different regions like Federal District, Lara state, Guacharo's cave in Monagas state and Miranda state.^{1,14}

The aim of this study was to assess the presence of primary pathogen fungi in dove's excreta in Bolívar state, Venezuela.

MATERIAL AND METHODS

Geography and population

Bolívar State has a surface of 240528 Km², and a population of 3.8 inhabitants/km², distributed mainly in Caroní (51.7%) and Heres (25.6%) counties. Ciudad Bolívar, the

* Departamento de Parasitología y Microbiología, Escuela de Medicina "Dr. Francisco Battistini Casalta".

** Departamento de Medicina, Hospital Universitario "Ruiz y Páez". Universidad de Oriente, Ciudad Bolívar, Venezuela.

state capital at the southeast of Venezuela, is an Orinoco river port in Heres county. It is situated between 0.3° 35' and 8° 27' north latitude and 60° 17' and 67° 27' west longitude (Greenwich). It has a tropical rainy weather followed by a very dry season. Its annual range temperature is 23.7°C to 28.2 °C, the maximal temperature is 33.1°C to 34.1°C and minimal temperature of 23.7°C to 24.3°C. Its annual rainfall is 820-1326 mm; however pluviosity is variable. Gallery woods on rivers banks, semi deciduous shrubs on the savannas, with groves of "Moriches" (a tropical palm) and chaparrals on the river banks are observed.^{22,29}

Samples

From June 2000 to August 2002, dove's excreta samples were collected in different places at Bolívar state. Selection of collection sites was made looking for dove gathering places and reports of inhabitants about them. In each place, 10 to 100 g of dove's excreta were collected, using a little sterile wood shovel, in a plastic bag with a tight seal. Samples were carried to laboratory and processed the same day. The number of doves at the moment of sample collection, excreta appearance and accumulation degree, classified as scarce (1 or 2 layers of excreta) or abundant (more than 2 layers), were registered for each place. A measurement of excreta pH was made.

Samples processing

Samples were processed according to Shields and Ajello technique modified by Pal and Baxter.^{2,3,34} Briefly, dove's excreta suspensions were made in sterile saline solution 1:10 (w/v). Chloramphenicol 0.3 g/l was added and pH was measured. Suspensions were stirred during 5 minutes and were allowed to settle for 30 minutes. An aliquot (0.1 ml) of supernatant from each suspension was inoculated in duplicated in Petri dishes of *Guizotia abyssinica*-agar and sunflower (*Helianthus annuus*) seed agar media. Plates were incubated in duplicated for 30 days at 30°C and daily examined. Brown colonies suspected being *C. neoformans* were compared to a control colony. From each suspected colony, a sample was studied microscopically using lactophenol blue and black ink stains. Identification of yeasts was performed using urea hydrolysis test, sugars and nitrates assimilation, resistance to actidione phenyloxidase test, germ tube test, clamidospore on appropriate agar, colonies on Staib agar, growth in Sabouraud agar at 45°C and sugar assimilation test (Api 20C Aux-Biomerieux and Auxacolor Sanofi Diagnostics Pasteur). For *Cryptococcus* variety, determination L-canavanine, glycine, bromothymol blue, proline and tryptophan assimilation tests were used.^{4,8,18-20,23,30}

When mycelium growth was observed, its microscopic morphology was determined using lacto phenol cotton blue stain, 10% KOH examination and microcultures in slides and tube culture in Sabouraud, glucose-potato-agar and blood-agar with antibacterial drugs (Gentamycin, Chloramphenicol and Cicloheximide).

H. capsulatum dimorphism was shown by culture incubation at 28°C and 37°C during 8 weeks.^{3,18}

Statistic analysis

Chi square and Fisher exact tests were performed using the Statistical Package for Sciences for Windows, 7.2 version, a significance level of $p < 0.05$ was chosen.

RESULTS

A total of 116 dove's excreta samples were collected from several places in Bolívar state: Ciudad Bolívar (n=36; 14.6%); Puerto Ordaz (n=15; 12.9%), San Félix (n=26; 22.4%), Guasipati (n=13; 11.2%) and Tumeremo (n=26; 22.4%). Degree of accumulation was abundant in 66.3% (n=77). The number of doves found at collection site ranged from 0 to 330 with a median of 4; there was no relationship between number of doves and fungus isolation. Excreta were dry in 87.0% (n=101). Excreta pH was between 6 and 8, being pH=7 in 77.7% (n=80). Twenty samples (68.9%) were collected from open places. There was a statistically significant relationship ($p=0.04$) between pH 7 and isolation of yeasts and mould.

In incubated dishes, many colonies of saprophyte bacteria, yeasts and moulds were observed at 48 to 72 hours of incubation at 37°C. Yeasts were isolated in 15.1% (n=11) of samples, being more frequently found *C. albicans* (4.1%; n=3), *C. dubliniensis*, *C. guillermoidii* (1.4%; n=1) and *Cryptococcus albidus* (2.7%; n=2). *C. neoformans* var *neoformans* was isolated in one sample (1.4%) proceeding from an open place, of high degree of accumulation, dry and pH 7, at Complejo Universitario "Ruiz y Páez" vicinity in Bolívar City. *C. neoformans* started to grow at the 4th day of incubation.

Growth of multiple filamentous fungi was observed and 74 moulds were isolated. The most frequently found fungi were *Aspergillus* spp (31.1%; n=23), *Mucor* spp (20.2%; n=15), and *Fusarium* spp (6.7%; n=5) (Table 1). *H. capsulatum* was isolated in one sample (1.3%) from a closed and wet place, with 3 doves, great accumulation of excreta and pH 7, in Bolívar City.

DISCUSSION

C. neoformans and *H. capsulatum* were isolated in dove's excreta only in Bolívar City at Bolívar state, there-

Table 1. Frequency of filamentous and yeast-like fungi in dove's (*C. livia*) excreta.

Bolívar State (Venezuela)		
Fungi	n	
Yeasts		
<i>Candida albicans</i>	3	(4.1)
<i>Candida dublinensis</i>	1	(1.4)
<i>Candida guilliermondi</i>	1	(1.4)
<i>Cryptococcus albidus</i>	2	(2.7)
<i>Cryptococcus neoformans</i> var <i>neoformans</i>	1	(1.4)
<i>Rhodotorula</i> spp	2	(2.7)
<i>Trichosporum asahii</i>	1	(1.4)
Subtotal	11	(15.1)
Filamentous		
<i>Alternaria</i> spp	5	(6.7)
<i>Aspergillus</i> spp	23	(31.1)
<i>Curvularia</i> spp	2	(2.7)
<i>Fusarium</i> spp	5	(6.7)
<i>Histoplasma capsulatum</i>	1	(1.3)
<i>Trichophyton</i> spp	3	(4.1)
<i>Microsporum</i> spp	1	(1.3)
<i>Mucor</i> spp	15	(20.2)
<i>Penicillium</i> spp	7	(9.5)
<i>Phoma</i> sp	1	(1.3)
Subtotal	63	(84.9)
Total	74	(100)

fore the presence of these primary pathogen fungi is an important finding since this shows the distribution of saprophytic form of these fungi in this region and possible sources for infection.^{9-11,16} The isolation of *C. neoformans* was done in a sheltered open place, as other authors have previously reported⁴¹⁻⁴³.

Some venezuelan cases of cryptococcosis have been described in different regions of this country standing out Federal District, Táchira, Zulia, Monagas, Miranda and Bolívar states as endemic regions^{9,40,41}. Isolation of *C. neoformans* has been described in bird's excreta from squares and public fountains in Caracas⁴¹. The high accumulation of dove's excreta is related to presence of fungi and it has been suggested that appropriate environmental conditions are a necessary factor for preservation of fungi in nature.^{7,12,20} In other regions, cases of cryptococcosis have occurred in the area where *C. neoformans* had been isolated from the environment and it has been suggested that epidemiological studies of clinical isolates and environmental isolates should be important to determine origin of infection and the route transmission.⁴²

C. neoformans has been isolated in vegetable soil, fruit skin, milk, wild and domestic animals, and bird's dejections, specially of doves.^{2,13,15,17,21,25,31,32,35,36} In this study, there was not association between number of doves at samples collection site and isolation of fungus. However, it has been shown a relationship between the presence of bird's excreta and multiplication of fungi in them.^{13,17,21,32,35,37} Ecology of *H. capsulatum* has been widely studied in the last decade and it has been considered that is very likely to be in soils with bats or birds excreta and therefore it is frequently isolated from caves, mines, farms, woods, old houses or any place where these animals live.^{6,24}

Ciudad Bolívar is situated in a tropical zone, with appropriate highness and humidity in addition to soil natural substances with high content of nitrogen (excreta) that ensure a propitious ecological niche for development of primary pathogen fungi like *C. neoformans* and *H. capsulatum* in the environment. Also, saprophyte fungi found in soil and dove's excreta have been shown in this and other studies.^{13,15,21,26,32,35,37} Several authors have shown that cryptococcosis and histoplasmosis are endemic in Bolívar state.^{10,11,16}

This study has shown the presence of *C. neoformans* and *H. capsulatum* in Bolívar State, particularly in Bolívar City, and it remarks the need to avoid accumulation of dove's excreta in the city due to the contamination risk with these pathogens fungi.

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Correspondence to:

Dr. Julman R. Cermeño.

Avenida 17 de Diciembre
Centro Comercial Country. Local 3. Piso 1.
Ciudad Bolívar. Estado Bolívar. Venezuela.
P.O. Box 222, Ciudad Bolívar 8001, Venezuela.
Tel-Fax: + 58- 2-85 6543291.
E-mail: jcerme@cantv.net; jicervi@yahoo.com;
spenna@cantv.net