
Epidemiological study of paracoccidioidomycosis and histoplasmosis in a suburb of San Félix city, Bolívar state, Venezuela.

Julman Cermeño¹, Julmery Cermeño¹, Gerardo Godoy¹, Isabel Hernández¹, Yida Orellán¹, Ytalia Blanco¹, Salvador Penna¹, Luily García², Thamara Mender¹, Marisa Gonsálvez¹, César López², Neudys Hernández², Isabel Longa², Esther Gottberg², Amarilys Basanta², Milagros Castro², Iraida Millán², Walesca León², Flor Plas², Caroline Jahouhari¹ and Ismery Cabello¹.

¹Escuela de Ciencias de la Salud “Dr. Francisco Battistini Casalta”, Universidad de Oriente Núcleo Bolívar, Ciudad Bolívar y

²Hospital “Raúl Leoni”. San Félix, estado Bolívar, Venezuela.

Key words: Epidemiology, histoplasmosis, paracoccidioidomycosis, Venezuela.

Abstract. Epidemiologic studies of deep mycosis have been scarce in Bolívar state, where paracoccidioidomycosis and histoplasmosis are considered as endemic diseases. The aim of this study was to determine paracoccidioidomycosis and histoplasmosis prevalences in people from a suburb of San Félix, Bolívar state, Venezuela. Three-hundred volunteers agreed to participate in this study and they were inoculated with paracoccidioidine and histoplasmine. Identification and epidemiologic data were registered. Reading of skin tests after 24 hours was performed in 275 persons. Paracoccidioidine test was positive in 10.2% (n=28). A higher percentage of positive reactions in the age group of 40-50 years old (n=10; 35.7%) was observed. Bricklayers, farmers and miners were positive in 27.3 % (3 out of 11), a higher percentage than in people with other occupations. Histoplasmine test was positive in 7.6% of cases (n= 21). The higher percentage of reactivity was observed in the age group of 40-50 years old (n=9; 42.9%). There was a direct proportional relationship between staying time in the locality and *H. capsulatum* infection mainly in persons staying in the area for more than 30 years (p<0.05). These results showed low prevalences of *P. brasiliensis* and *H. capsulatum* infection in this area.

Estudio epidemiológico de la paracoccidioidomicosis e histoplasmosis en una población suburbana de San Félix, estado Bolívar, Venezuela.

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Palabras clave: Epidemiología, histoplasmosis, paracoccidioidomicosis, Venezuela.

Resumen. El estudio epidemiológico de las micosis profundas ha sido escaso en el estado Bolívar donde la paracoccidioidomicosis e histoplasmosis son endémicas. El objetivo de este estudio fue determinar la prevalencia de infecciones por *Paracoccidioides brasiliensis* e *Histoplasma capsulatum* en individuos residenciados en una población suburbana de San Félix, estado Bolívar, Venezuela. Se administró paracoccidioidina e histoplasmina a 300 personas voluntarias. Se realizó lectura de las pruebas a las 24 horas. La paracoccidioidina fue positiva en el 10,2% (n=28). Se observó mayor porcentaje de positividad en el grupo de 40-50 años (n=10; 35,7%). Las ocupaciones de riesgo: albañiles, agricultores y mineros, presentaron un porcentaje de positividad de 27,3% (3 de 11), mayor que el de los individuos sin riesgo aparente: mecánicos, oficios del hogar y estudiantes (25 de 264; 9,5%) (p=0,04). La histoplasmina fue positiva en el 7,6% (n=21). El mayor porcentaje de intradermorreacción positiva se observó entre los 40-50 años (n=9; 42,9%). Hubo relación significativa entre el tiempo de residencia en la localidad y la infección por *H. capsulatum*, demostrándose en los individuos con más de 30 años en esa localidad (p<0,05). Estos resultados muestran una prevalencia relativamente baja de infecciones por *P. brasiliensis* y de *H. capsulatum* en el área estudiada.

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INTRODUCTION

Paracoccidioidomycosis (PCM) and histoplasmosis (H) are systemic mycosis produced by geophilic dimorphic fungi *Paracoccidioides brasiliensis* and *Histoplasma capsulatum*, respectively (1-3). These mycosis are endemic diseases in the south of Venezuela and are considered as a public health problem (4, 5). They are probably related to environmental conditions like humidity, temperature and soil characteristics (10-28°C, pluviometry 500-2500 mm/year, acid soils with rivers around for *P. brasiliensis* and 22-29°C, pluviometry 1200 mm/year, humidity 67-87 % and soils with

high organic content for *H. capsulatum*) that determine the distribution of these fungi in nature (1, 6-10).

PCM is a chronic progressive infection with a geographic distribution limited to the Americas, specifically the Latin American countries. It is characterized by a primary lung infection, frequently unapparent, which can disseminate, principally presenting lesions on the mucosae of the upper airways and upper digestive tract and occasionally the gastrointestinal tract. The lymph nodes are frequently involved and occasionally the disease extends to other organs, especially the skin tissue and adrenal glands (11).

Histoplasmosis is almost always acquired by inhalation of the mycelial fragments and microconidia of the organism from an environmental source, most commonly disturbed earth or bird or bat droppings. The organism is found worldwide and it is endemic in parts of the United States, South America, Southeast Asia, Africa, and Australia. Usually H presents as a disseminated infection with fever and weight loss. Respiratory complaints, local or generalized lymphadenopathy, hepatosplenomegaly, colonic lesions, and skin and oral ulcers may also be present. Diagnosis is usually made by culturing the fungus from blood or other clinical specimens, or by histopathologic examination of bone marrow aspirate or biopsy material (9, 12).

Intradermic tests like paracoccidioidine and histoplasmine are often used in epidemiologic studies in order to detect PCM and H respectively (6, 13, 14), particularly in cases of asymptomatic infections. In these tests, fungus antigens are intradermically injected to induce a reaction that is read 24 and 48 hours later and a positive reaction is an indurated area of 5 mm or more. A positive reaction suggests the presence of fungus infection (13, 15-17).

Epidemiologic studies of deep mycosis have been scarce in Bolívar state, even though PCM and H are considered as endemic diseases (5, 18) and, therefore, it is necessary a better knowledge of the clinical and epidemiologic aspects of these diseases.

Most of cases of PCM and H in children that have been diagnosed in "Raúl Leoni" Hospital in San Félix, Bolívar state, came from the "25 de Marzo" community, for that reason this place was chosen to realize this epidemiologic study in order to determine prevalence of *P. brasiliensis* and *H. capsulatum* infections in people living in this zone.

PATIENTS AND METHODS

Geographic characteristics

This study was performed in the community "25 de Marzo", a suburban area with 46309 inhabitants (19). It is localized in Caroni county, San Felix city, situated in the north-east region of Bolívar state in south-east of Venezuela. It has a mean annual precipitation of 1000 mm³ and a relative humidity of 70% (20).

Samples

A group of 300 persons voluntarily agreed to participate in this study. Pregnant women, children under 6 months old, adults older than 65 years old and people with a history of systemic mycosis were excluded. Identification and epidemiologic data was registered.

Clinical and laboratory evaluation of symptomatic patients

All patients with respiratory symptoms were clinically evaluated and a radiological study of the thorax was made. Sputum samples were collected in patients with a probable diagnosis of PCM and/or H. All secretions were evaluated with Ziehl Neelsen stain, KOH 20% and argentic methenamine stain. All samples were cultured, by duplicate, in glucose agar Sabouraud and heart-brain agar; they were incubated at 37°C during 8 weeks. In addition, antibodies against *H. capsulatum* and *P. brasiliensis* in serum samples were determined.

All samples were processed in the Parasitology and Microbiology Department, Universidad de Oriente, Núcleo Bolívar.

Serological study

Before cutaneous tests were performed, blood samples were taken for serology. To demonstrate specific antibodies against *P. brasiliensis* and *H. capsulatum*, GP43 antigen of *P. brasiliensis* (gently sup-

plied by Dr. Zoilo Pires de Camargo, Sao Paulo, Brasil) and *H. capsulatum* antigen from Meridian Bioscience Inc. (Cincinnati, Ohio, USA, 45244) were used. Immunodiffusion test in agarose gel was performed as advised by the manufacturer. Detection of H and/or M band was considered positive for *H. capsulatum* and presence of any identity band was considered positive for *P. brasiliensis*.

Cutaneous tests

P. brasiliensis and *H. capsulatum* var *capsulatum* antigens were used and they were made in the Institute of Biomedicine, Universidad Central de Venezuela, Caracas.

Paracoccidioidine is a polysaccharide obtained from the yeast phase of 8-9 isolates of *P. brasiliensis*. Histoplasmine is a metabolic antigen obtained from the filamentous phase of *H. capsulatum* (21). Cutaneous tests were realized after extraction of blood samples to determine specific antibodies against *P. brasiliensis* and *H. capsulatum*. During the test, 0.1 mL of paracoccidioidine was intradermally injected in the anterior face of the proximal third of left forearm and 0.1 mL of histoplasmine in the anterior face of the proximal third of the right forearm of each person, simultaneously. Inoculation area was identified with a mark. Disposable syringes of 1 mL and 27 gauge needles were used.

Readings were made only at 24 hours after the injections because the subjects accepted to participate in the study during only two days: the first day, when clinical data would be collected and cutaneous tests would be applied; and the following day, when the readings would be realized; as at 24 hours many subjects have already reacted to these cutaneous tests (22), we considered the results would be valid. The tests were considered positive when an endured and edematous area with a diameter of 5 mm or more in the place of inoculation

was developed (16, 17). Positive reactions were considered indicative of past, intercurrent or subclinical infection by *P. brasiliensis* or *H. capsulatum*.

Statistical analysis

Chi-square test and exact test of Fisher were performed with a significance level of $p < 0.05$ using SPSS program, version 7.5 for Windows.

RESULTS

Serological study

Specific antibodies against *H. capsulatum* (H and M bands) were not demonstrated in any patients. Only in a case diagnosed as PCM, specific antibodies against *P. brasiliensis* were detected.

Paracoccidioidine intradermic test

A group of 300 intradermic tests was performed, but in 25 individuals (8.4%) the results could not be evaluated. Two hundred seventy five persons with a mean age of 17.3 ± 15.6 years old, in a range from 7 months to 63 years old, were evaluated; the largest age group was under 10 years old ($n=129$; 46.9%). Feminine gender was represented by the 61.5%. The 86.9% ($n=239$) of evaluated persons came from "El Progreso" ($n=129$; 46.9%), and "El Porvenir" zones ($n=110$; 40%), with a mean of 7.6 ± 8.4 years living in those places (range 1 month to 46 years). 52% ($n=143$) was unemployed and 20% ($n=57$) was homemaker.

The intradermic test was positive at 24 hours in 10.2% ($n=28$) of them. Reactivities to paracoccidioidine by age groups are in Table I. The age group of 40-50 years old was the group with the highest percentage between paracoccidioidine reactors ($n=10$; 35.7%) and feminine gender (71.4%; $n=20$) had the highest percentage of positivity.

High risk workers like bricklayers, farmers and miners showed a percentage of

positivity (27.3%; 3 out of 11) significantly higher than that in persons without an apparent risk like mechanics, houseworkers and students (9.5%; 25 out of 264) ($X^2 = 3.94; 1 \text{ df}, p = 0.04$). See Table II. Time living in the area was not associated with positivity in the test.

In the group with positive intradermo-reaction, 6 (21.4%) were smokers, 14 (50%) non smokers and 8 (28.5%) ex-smokers.

Collateral effects of the test were only observed in 8.4% (n=23), the most frequent were erythema (n=6; 26.1%) and pruritus (n=6; 26.1%); less common were general malaise, edema, low fever, sore throat and local burning. With regard to chronic respiratory symptomatic patients,

39 patients were evaluated and only one case was diagnosed with PCM by demonstration of the fungus in sputum stains and culture. In addition, specific antibodies in serum were detected. Clinical features were chronic cough, loss of weight and mucous expectoration. Bilateral diffuse infiltrates were observed in the thorax radiography. This patient was treated with Itraconazole 200 mg/day and periodic control.

Histoplasmine intradermic test

In a similar way, this test was given to 300 persons and the reading at 24 hours afterwards could only be realized in 275 individuals. The intradermic histoplasmine reaction was positive in 7.6% of cases (n= 21).

TABLE I
 REACTIVITY TO PARACOCCIDIOIDINE AND HISTOPLASMINE BY AGE GROUPS
 IN THE "25 DE MARZO" POPULATION, SAN FÉLIX, BOLIVAR STATE, VENEZUELA

Age groups (years)	Paracoccidioidine	Histoplasmine
0-10	7* (5.4)/129	2 (1.6)/129
11-20	1 (2.0)/50	2 (4.0)/50
21-30	3 (10.3)/29	0/29
31-40	5 (16.1)/31	4 (12.9)/31
41-50	10 (35.7)/28	9 (33.3)/27
51-60	0/5	2 (40.0)/5
> 60	2 (50.0)/4	2 (50.0)/4
Total	28 (10.2)/275	21 (7.6)/275

*Number of reactor/Numbers of tested subjects. Values in parenthesis are percentages.

TABLE II
 RELATIONSHIP BETWEEN REACTIVITY TO PARACOCCIDIOIDINE AND HISTOPLASMINE
 AND OCCUPATIONAL OR RECREATIONAL EXPOSITION IN THE "25 DE MARZO" POPULATION,
 CIUDAD BOLÍVAR, BOLÍVAR STATE, VENEZUELA

Occupational or recreational exposition	Paracoccidioidine*			Histoplasmine**		
	Positive n (%)	Negative n (%)	Total n (%)	Positive n (%)	Negative n (%)	Total n (%)
Yes	3 (10.7)	8 (3.2)	11 (4.0)	4 (19.0)	7 (2.7)	11 (4.0)
No	25 (89.3)	239 (96.7)	264 (96.0)	17 (80.9)	247 (97.2)	264 (96.0)
Total	28 (10.2)	247 (89.8)	275 (100)	21 (7.6)	254 (92.4)	275 (100)

* $X^2 = 3.94$. 1 df. $p = 0.04$. ** $X^2 = 13.407$. 1 df. $p = 0.006$. n=number of individuals.

Reactivities to histoplasmine by age groups are in Table I. The age group of 40-50 years old was the group with the highest percentage between histoplasmine reactors (n= 9; 42.9%), followed by the age group 30-40 years old (n=4; 19.0%). The 61.9% (n=13) of histoplasmine reactors were feminine.

A direct proportional relationship between the time living in this place and the presence of *H. capsulatum* infection was statistically significant, particularly in persons living there for more than 30 years ($X^2=27.86$; 2 df, $p<0.0001$).

There was no evidence of a significant relationship between contact with birds or fowls or visiting caves and positivity to histoplasmine test. However, a significant association between occupational risk (farmers) and *H. capsulatum* infection was shown (see Table II) (Exact test of Fisher, $p= 0.006$).

DISCUSSION

Intradermic tests like paracoccidioidine and histoplasmine have been very useful in epidemiologic studies to investigate prevalence of subclinical infection of *P. brasiliensis* and *H. capsulatum* in endemic areas (14, 17, 23). Furthermore, these tests are simple to use and easily evaluate the immune response, but are not diagnostic by themselves. In this study we found 12 cases (4.4%) positive to both tests. Thus, it may correspond to a cross-reaction with either other primary pathogens, saprophytes or between each other, a common feature in intradermic tests (10, 13, 16, 21).

In this study, a higher proportion of persons reacting to paracoccidioidine (10.2%) than to histoplasmine (7.6%) was found, differing from studies carried out in other zones of Bolivar state, where positivity was higher to histoplasmine: 8.5% and 11.3% at 24 hours for paracoccidioid-

ine and 25.7% and 19.6% at 24 hours for histoplasmine (4,18). This difference could be explained by the presence of rivers and lagoons around this area, a favorable environment for the development of *P. brasiliensis* (10).

However, frequency of positivity to paracoccidioidine was lower than in other endemic regions of Venezuela, i.e. Sierra de Perijá (Zulia state), Paracotos (Miranda state), Culebra island (Carabobo state) and Sierra de San Luis (Falcón state), with a positivity of around 60% (15, 16, 24, 25) and than other endemic Latino American regions: Chaco province of Argentina (26) and Serra de Pereiro, Ceará, state of Brasil, where 32.1% of positivity was found (13). Although, in this study, the reactivity to both antigens might have been higher if the reading had been done at 48 hours, as has been found in other study (18).

In endemic areas of Central América, a high prevalence of histoplasmine reaction ranging from 12% to 40.4% has been observed in persons who have visited caves recently (3, 27, 28): 12% in Martinica (23) and between 13.2%-28.8% in Cuba (6, 29), higher than the prevalence found in this study. This difference might be in relation with ausence of caves in this area and the early reaction reading, before a posible peak of reactivity at 48 hours (18).

Children infection in endemic areas is high (10). Young adults and children are considered as epidemiologic markers since they may have less chance to be in contact with fungus (16,30). Thus, the finding of people under 20 years old with positive intradermic reaction could be an indicator of the presence of fungus in the soil in the studied area.

It is pointed out that precocious infection in children under 10 years old by *P. brasiliensis* (1.6%) and *H. capsulatum* (5.4%) is not negligible, although it is much lower than that found in zones of Ar-

gentina where infection rate ranges between 9.2% and 53.5% (26,31).

People of any age are susceptible to *H. capsulatum* and *P. brasiliensis* infection. Nevertheless, some authors have suggested that positivity of intradermic reaction to fungus antigens increases with age (28), like our results where people older than 40 years represented more than 40% of positives cases in both entities. High frequency of reactivity in feminine group, in both cutaneous tests could be well explained by the bias given by gender distribution of the studied population where the feminine group was predominant.

Ausence of antibodies against *P. brasiliensis* and *H. capsulatum* in the population infected but not ill, was as expected, since these antibodies are diagnostic of those diseases.

Our results showed a low prevalence of *P. brasiliensis* and *H. capsulatum* in the studied area; in addition, the subclinical infection index in children and housewives indicates that exposition to these fungi and the possible coexistence of *H. capsulatum* and *P. brasiliensis* in that area occur.

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