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Paracoccidioidomycosis in Colombia: an ecological study

D. CALLE[1], D. S. ROSERO[1], L. C. OROZCO[2], D. CAMARGO[2], E. CASTAÑEDA[3] AND A. RESTREPO[4]*

[1] *Facultad de Medicina, Universidad Pontificia Bolivariana, Medellín*
[2] *Universidad Industrial de Santander, Bucaramanga, Colombia*
[3] *Instituto Nacional de Salud, Bogotá, Colombia*
[4] *Corporación para Investigaciones Biológicas, Medellín, Colombia*

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SUMMARY

The natural habitat of *Paracoccidioides brasiliensis*, agent of paracoccidioidomycosis (PCM), remains unknown. This study is aimed at establishing associations between the ecological variables present in all Colombian municipalities and the incidence of PCM. Records of 940 patients were studied and several ecological variables analysed, as well as their association to amount of patients per total rural population in each municipality, determined through a multivariate analysis. All 940 patients came from 216 municipalities (20·3 %), out of which, 93 were birthplace and place of long-term residence for 121 patients. The Incidence Rate Ratio (IRR) was determined for these 93 municipalities. The following variables fitted the model: altitude from 1000 to 1499 metres above sea level (IRR = 6·37), rainfall from 2000 to 2999 mm (IRR = 2·15), presence of humid forests (Holdridge) (IRR = 1·79) and coffee (IRR = 1·95), tobacco (IRR = 3·59) crops. These results indicate that these municipalities constitute *reservareas* for *P. brasiliensis* (Borelli).

INTRODUCTION

Paracoccidioidomycosis (PCM) is a systemic mycosis of singular importance in Latin American countries. It is caused by *Paracoccidioides brasiliensis*, a thermally dimorphic fungus with an undefined habitat [1–3].

This mycosis extends from Mexico (23° North) to Argentina (35° South) and is more prevalent in South than in Central America [4–6]. The countries registering the largest number of patients are Brazil, Venezuela and Colombia. Brazil is the centre of the endemic area [4, 7] and presents an annual incidence rate from 10 to 30×10^{-6} and a mean mortality rate of $1{\cdot}4 \times 10^{-6}$. In Colombia, Castañeda and colleagues [8] found that during the 1984–94 period the incidence fluctuated from 0·5 to 2·2 per $\times 10^{-6}$ inhabitants, respectively.

PCM is characterized by long periods of latency and consequently the endemic area may not correspond to the fungus natural habitat [2, 3]. On this basis, Borelli created the term *reservarea* to indicate that particular site where both circumstances, infection and natural habitat, coincide [9].

Several factors have hindered finding *P. brasiliensis*' natural habitat, among them the long periods of latency, the fact that PCM is not a compulsory reportable disease and, also the absence of both outbreaks and acute cases [2, 5].

Previous publications have dealt with ecological characteristics that appear to favour *P. brasiliensis*' development in nature, all of which refer to the following factors as of particular interest: an air temperature between 17 and 24 °C [9–14], an annual

* Author for correspondence: Corporación para Investigaciones Biológicas, Carrera 72A, no. 78B-141, Medellín, Colombia.

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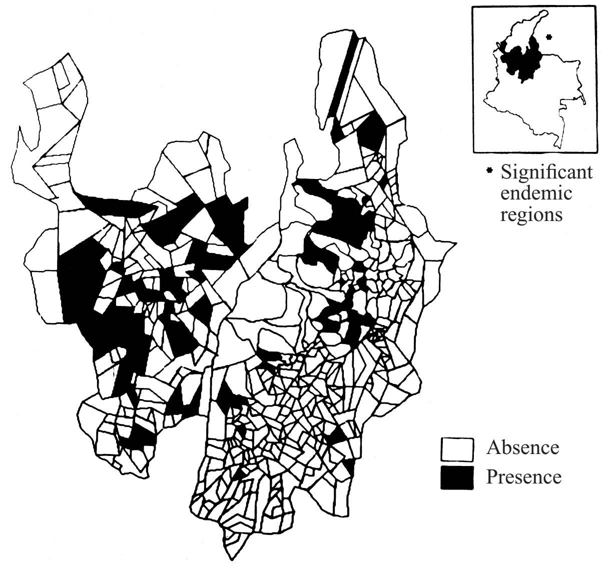

Fig. 1. Colombian municipalities. Distribution of paracoccidioidomycosis according to presence or absence of the disease within the most significant endemic region. The municipalities in black represent the *reservareas* for paracoccidioidomycosis in Colombia.

rainfall oscillating between 500 and 2500 mm [9–14], an altitude of 400–1200 m above sea level [9, 10, 12, 14–18], a high (60–70%) and constant relative humidity [12, 18], and presence of humid forests, tropical, subtropical or lower mountainous (Holdridge classification) [12, 13, 15, 17]. Other factors mentioned are soil pH, usually in the acidic range [5, 13, 15, 16, 18], presence of watercourses in the area [12, 16, 18], and existence of certain crops such as coffee and sugar cane [12, 15, 18].

Ecological studies are focused on a specific geographical area (country, state, county or municipality) which is then taken as analysis unit. These studies attempt to establish a relationship between different risk factors and a particular event for which the corresponding information is either non-available or has no significance at the individual level [19–22].

The aim of the present study was to determine, based on the incidence rates of PCM in Colombia, if certain ecological factors in some municipalities (geographic divisions in Colombia) corresponded to *P. brasiliensis*' natural habitat.

METHODS

Type of study

This was a mixed ecological study; ecological because it had the municipality as the unit of analysis, and mixed as it had, simultaneously, an exploratory character as well as a group comparison design [19–22].

Cases and case definition

Available clinical records of Colombian patients diagnosed as having PCM were reviewed in two mycoses reference centres: Instituto Nacional de Salud (INS), Santafé de Bogotá, and Corporación para Investigaciones Biológicas (CIB), Medellín. Additionally, the pathology registries at Hospital Universitario San Vicente de Paul, in Medellín, and Hospital Ramón González Valencia, in Bucaramanga, as well as the records of the Health School diagnostic unit at Universidad de Santander (UIS), were consulted. Case definition was based on demonstration of the aetiologic agent by direct methods such as KOH, biopsy and/or isolation in culture. Presence of serum antibodies against *P. brasiliensis* was also considered of diagnostic value.

Inclusion criteria

To become part of the study, clinical records had to include data corresponding to patients' birth and/or residence place; additionally, the diagnosis had had to be established not earlier than 1970. The criterion to accept residence municipality in rural area as significantly related to the mycosis, was the patient's permanent residence in the place for at least 3 years before diagnosis. This period was chosen on the basis of the CIB's clinical records that indicated this was the mean duration of the symptoms before diagnosis [23].

Population

Municipalities' rural population was taken from the 1985 official census established by the National Administrative Department for Statistics (DANE) [24]. This year is halfway through the study period which extended during 1970–99. The total rural population was then multiplied by 30 in order to obtain the number of person per year figure, which was used as incidence rate denominator (IR) [25]. The municipalities' names and codes used in the data base were taken from DANE's official publication [26].

Variables

The incidence rate (IR) was taken as the study's dependent variable and was calculated for those municipalities corresponding to patients that had been born and also been long-term residents there.

Table 1. *Bivariate analysis: incidence rates in municipalities that had been both place of birth and long-term residence of paracoccidioidomycosis patients*

Ecological characteristics prevailing in municipalities	Incidence rate*	IRR† (95% CI)
Altitude (m)		
0–499	0·106	‡
500–999	0·648	6·07 (2·84–12·96)
1000–1499	1·174	10·98 (5·75–21·00)
1500–1999	0·554	5·19 (2·58–10·42)
⩾ 2000	0·205	1·92 (0·82–4·52)
Precipitation (mm)		
< 2000 or ⩾ 3000	0·310	‡
⩾ 2000–2999	0·746	2·4 (1·66–3·49)
Humid forests (Holdridge)		
Absence	0·245	‡
Presence	0·561	2·28 (1·47–3·68)
Very humid forest		
Absence	0·226	‡
Presence	0·557	2·47 (1·54–4·12)
Coffee		
Absence	0·141	‡
Presence	0·729	5·19 (3·16–8·97)
Tobacco		
Absence	0·398	‡
Presence	1·110	2·79 (1·59–4·63)
Yucca		
Absence	0·354	‡
Presence	0·513	1·45 (0·99–2·14)
Sugar cane		
Absence	0·168	‡
Presence	0·708	4·21 (2·65–6·96)

* Persons/year $\times 10^{-6}$.
† Incidence Rate Ratio (IRR).
‡ Basis for comparisons.

The independent variables examined were the following: altitude which was expressed in metres above sea level, air temperature in Celsius, rainfall in millimetres (mm), type of crops which were limited to the 20 most commonly grown products [27, 28], and life zones based on the Holdridge system [17, 27–29]. Data for temperature and rainfall were expressed as mean per year.

The variables corresponding to the Holdridge life zones [29, 30], were obtained by projection of the corresponding maps over the official 1996 Colombian map prepared by DANE. In past publications, the life zones corresponding to the humid, very humid and lower mountainous forests have been shown to be associated to PCM [12–15, 18]. Consequently, such forests were the focus of attention in this study.

Databases and analysis

Microsoft Excel 7.0 was used to construct the databases. Incidence rate ratios (IRR) were calculated using Poisson's regression following Greenland's recommendations, a multivariate analysis, which was done using Stata 6 [31–33]. The maps were prepared in Epi-map 2 [34].

RESULTS

The search for PCM patients revealed that during the study period (1970–99), 940 cases had been registered in the four centres under study, 363 (38·6%) of which complied with our case definition requirements. Inclusion criteria were not fulfilled in the remaining 577 cases (61·4%). Among the 363 cases chosen, 328

Table 2. *Ecological variables according to Poisson's multivariate analysis for municipalities where place of birth and long-term residence coincided*

Ecological characteristics prevailing in municipalities	IRR*	$P >$	CI (95%)†
Altitude (m)			
0–499	1		
500–999	3·8	0·001	1·68–8·66
1000–1499	6·3	0·000	3·09–13·11
1500–1999	2·8	0·009	1·30–6·08
⩾ 2000	1·7	0·210	0·73–4·10
Precipitation (mm)			
< 2000 and ⩾ 3000	1		
2000–2999 mm	2·1	0·000	1·48–3·12
Presence of humid forests	1·7	0·010	1·15–2·80
Presence of coffee crops	1·9	0·024	1·09–3·49
Presence of tobacco crops	3·5	0·000	2·17–5·94

* Incidence Rate Ratio (IRR).
† 95% confidence interval.

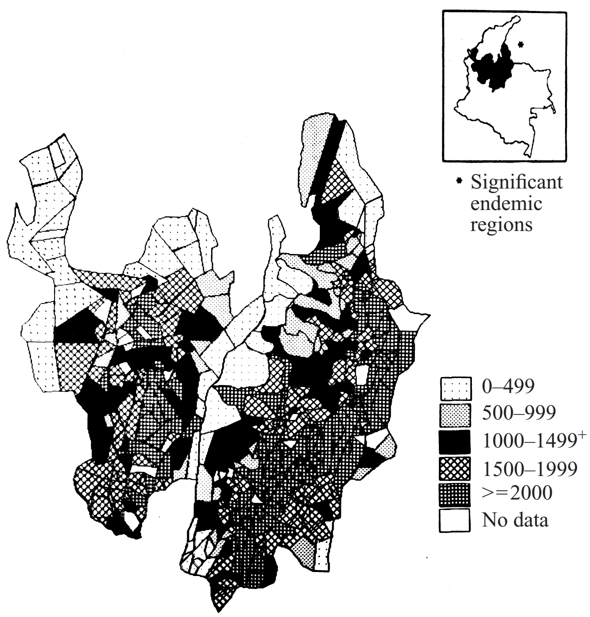

Fig. 2. Altitude (metres above sea level) in the municipalities with the highest IRs for paracoccidioidomycosis within the most significant endemic region. The municipalities in black (+) have the highest IRR = 6·37.

(90·3%) had information on birth place and 253 (69·6%) on residence place; additionally, 121 (33·3%) of the patients in these two groups coincided in indicating a municipality that was both place of birth and long-term residence.

From the 1059 Colombian municipalities, 43 had to be excluded due to insufficient information on the ecological variables under consideration. In 24 of the 1016 municipalities analysed, there were no data on rural population and this prompted us to assign them the median value of the remaining municipalities, estimated in 6700 inhabitants (data not shown). Several analyses were done to verify if this figure influenced the results; however, calculations using 5000 and 10000 inhabitants gave very similar results, with only slight changes in the first or second decimals of the IRR. In the present analysis, 1016 (96%) municipalities were included.

The 940 PCM cases were all distributed in 216 (20·4%) municipalities. From these, 93 municipalities (43%) had been both place of birth and long-term residence. Figure 1 depicts the area encompassing the municipalities with the highest endemicity. Cases appeared to cluster in and around the Andean region with variable IR values, from 0·00 to 43·12 cases $\times 10^{-6}$ person/year.

The initial bivariate studies revealed that the following characteristics were to be included in the multivariate analysis: metres above sea level, medium annual precipitation, presence of humid and very humid forests, as well as coffee, tobacco, sugar cane and yucca crops. Table 1 illustrates the results of the bivariate study that utilized the IRs for the cases in which both place of birth and long-term residence were the same.

The multivariate analyses revealed significant associations among the following ecological factors and the incidence of PCM (Table 2).

Altitude. This variable presented a positive increase in its IRR with its maximum in the range 1000–1499 m above sea level (IRR = 6·37; CI, 3·09–13·11). The IRR showed a trend to decrease with

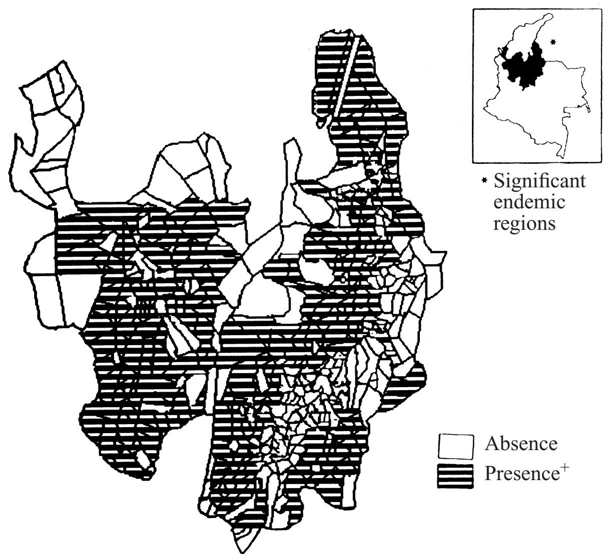

Fig. 3. Distribution of coffee crops (horizontal lines) within the most significant endemic areas for paracoccidioidomycosis in Colombia. The IRR in these municipalities (+) was high (IRR = 1·95).

increasing altitude, and around 2000 m values were no longer significant (Fig. 2).

Annual precipitation rates. These rates were significant in the 2000–2999 mm range (IRR = 2·15; CI, 1·48–3·12).

Presence of humid forests and crops. Important associations were also found between the presence of humid forest (IRR = 1·79; CI, 1·15–2·80); coffee (IRR = 1·95; CI, 1·09–3·49) (Fig. 3), and tobacco (IRR = 3·59; CI, 2·17–5·94) crops.

It was observed that in 6 municipalities with high incidence rates ($8{\cdot}1$–$30{\cdot}8 \times 10^{-6}$ person/year), 3–5 of the significant ecological variables were simultaneously present. When the 843 municipalities with no reported cases were compared to those with patients, only 21·4% of the former and 63·5% of the latter showed 3–5 significant variables simultaneously (data not shown).

DISCUSSION

Ecological studies are an important tool for establishing risk factors and generating hypotheses [19]. Orozco and Camargo [20] used this type of analysis and established associations between leprosy and altitude, as well as between population density and presence of cretaceous soils. By means of this methodology, Koopman was able to establish certain significant associations for dengue fever that could not be found at the individual level [35].

This is the first ecological study based in a multivariate regression analysis that explores environmental conditions prevailing in PCM endemic regions. Choosing the municipality as analysis unit allowed us to classify the PCM cases registered in a 30-year period in Colombia in each one of these units. We found that the 940 cases diagnosed during this period could be allocated to only 216 (23%) municipalities in the country. Among these, 93 (43%) corresponded to both place of birth and long-term residence of some patients. Such municipalities represent true *reservareas* as described by Borelli [9].

By using the IRs means of patients who never moved from their place of birth, it was possible to define these municipalities as both the *reservarea* and the endemic area. Additionally, the IRR analyses of either birth or residence place furnished similar figures to those presented above.

Several previous studies have suggested a relationship between PCM incidence and ecological variables. One of the first reports that clearly confirmed this was published by Bopp and Bernardi in 1967 [14], who described the geophysical aspects influencing incidence rates in the State of Rio Grande do Sul, Brazil. The 384 cases of the mycosis under study, revealed that their geographical distribution was not homogenous: in the State's northern part where forests and agriculture were abundant, the IR was substantially higher (6·6/100000 inhabitants) than in the southern region (1·7/100000) where vegetation was poor and pasture predominated [14].

In the present study having 121 patients whose place of birth and long-term residence corresponded to the same municipality allowed for the establishment of a more precise association between the IRs and the ecological variables. The highest IRs were the following: altitude between 1000 and 1499 m above sea level (IRR = 6·37), altitudes corresponding to a 19·6–22 °C temperature range [29, 30], rainfall range between 2000 and 2999 mm (IRR = 2·15) and presence of coffee (IRR = 1·95) and tobacco (IRR = 3·59) plantations. It is also worth mentioning that *P. brasiliensis* isolation from soil by both Albornoz and colleagues [36] in Venezuela and Silva-Vergara [37] in Brazil were done in coffee-growing areas.

Former studies by Borelli [9, 10], Restrepo and Espinal [18], showed that there was an association between the mycosis and altitudes between 800 and 2100 m above sea level. Additionally, a number of studies have pointed out that *P. brasiliensis* apparently prefers temperatures between 17 and 24 °C [9, 10, 15, 18]. Our findings match the ecological conditions previously mentioned, but they define optimal ranges for *P. brasiliensis* development in nature more precisely. Precipitation indexes in endemic areas have also been mentioned in the past, limiting precipitation to 2000 mm/year [9, 10, 12–16, 36]. Our data, however, show a higher precipitation range (2000–2999) as the optimal one (IRR = 2·15).

Previous data on the associations among ecological aspects and PCM have been derived from descriptive studies [9, 12–15, 18, 36, 37], with the exception of the work by Cadavid and Restrepo [16]. By means of logistical regression methods these authors found that residents with the highest infection rates in 3 of the 5 municipalities under study, have had frequent contacts with armadillos (*Dasypus* spp.) and had regularly used water from certain streams.

A study aimed at determining the life zones (as defined by Holdridge) where PCM patients had lived [18], suggested an association between the tropical humid and very humid lower mountain forests and presence of PCM cases. In the present study, however, only the first aspect adjusted to the multivariate analysis (IRR = 1·79), while the second lost its importance.

A large number of PCM endemic regions are located in coffee-growing which also share a number of ecological characteristics significant for PCM, such as altitudes ranging from 1300–1800 m above sea level, 2000 being the maximum, temperatures between 18 and 22 °C and presence of humid forests [27, 29]. Tobacco plants, however, require somewhat different ecological conditions, including a wider altitude range (0–2000 metres), higher temperatures (18–28 °C), and drier climates (913–1387 mm/year) [27, 29] although they share environmental conditions with humid forest regions [29]. Clarifying the importance of two slightly different situations in *P. brasiliensis* ecology requires further study. Nonetheless, it was interesting to discover that 63·5% of the municipalities with high incidence rates ($> 8{\cdot}1 \times 10^{-6}$/year) showed more than three significant ecological factors.

We may have chosen not to include confounding variables [32] in the present study. Nonetheless, when comparing our findings with those in previous descriptive studies, it was apparent that various ecological variables formerly associated to PCM, altitude for instance, were validated, more precisely defined or discarded as in the case of sugar cane plantations. If information on birth and residence place had not been registered properly for reasons other than administrative ones, this study could have suffered from a selection bias [38]. Nevertheless, and as mentioned before, collection of the corresponding information increased during the years of the study from 22 to 53%.

According to Susser, the exposure variables under considerably in this study were integral rather than to contextual [21]. They were gathered from institutions with a long record in the analysis of ecological data and consequently, the risk of error in their classification should have been low. If the bias existed, it would have been non-differential and the corresponding associations would have had tended to have a null value [25, 39].

The present study formerly addresses the issue of ecological associations in PCM and, as such, opens the way to further developments in the field. The results presented here were based on a strict definition of case and used multivariate analysis to determine risks after adjusting possible confounding variables.

Although it is now possible to define more precisely prevailing conditions in PCM *reservareas* in Colombia, further work is necessary. Thus, studies should be conducted in municipalities with higher IRs and presence of significant ecological factors (high IRRs) in order to analyse smaller and finer units of measure, such as boroughs, and thus define *P. brasiliensis*' microniche.

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