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Factors associated with *Paracoccidiodes brasiliensis* infection among permanent residents of three endemic areas in Colombia

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SUMMARY

The natural habitat of *Paracoccidioides brasiliensis*, the aetiologic agent of paracoccidioidomycosis, has not been determined. Consequently, the events leading to the acquisition of infection remain controversial. To identify factors associated with infection in endemic areas we conducted a survey in three rural communities in Colombia where we had previously diagnosed paracoccidioidomycosis in children. Permanent residents were surveyed taking into consideration environmental and occupational variables. Skin tests were used to classify subjects as infected or non-infected. Variables found associated with infection were: (i) community A: previous residence around Porce river and agriculture in vegetable gardens; (ii) community C: frequent use of specific water sources; (iii) community V: housekeeping activities, and (iv) total group: age > 25 years and contact with bats. Residents in communities with higher prevalence of infection were older, had more complex residence history, and referred more contact with armadillos than residents of communities with lower infection.

INTRODUCTION

The dimorphic fungus *Paracoccidioides brasiliensis* is the aetiologic agent of paracoccidioidomycosis (PCM), one of the most prevalent systemic mycoses in Latin America [1]. Unlike other systemic mycoses, for which the natural microniches of the aetiologic agents have been characterized [2], the precise habitat of *P. brasiliensis* remains elusive [3]. The consensus prevails that the habitat is the soil of endemic areas; however, isolation of *P. brasiliensis* from soil has been reported conclusively only once, when Albornoz [4] isolated the fungus from 3 of 87 samples. However, other workers who used identical techniques failed to recover the fungus from soils [3].

Another aspect that has hindered determination of the habitat is the prolonged latency period of PCM [5], which, in conjunction with the frequent migration of the inhabitants of the endemic areas, makes it difficult to locate the site where the infection was acquired. This, in turn, results in a situation whereby the place where the infection is recognized does not correspond to the site where the

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infection was acquired [5–7]. To help solve this problem, Borelli [8] created the term 'resevarea' to designate areas where the fungus occurs in nature and where the host acquires the primary infection.

Skin tests have revealed that the peak prevalence of positive paracoccidioidin reactions occurs in adults, with children and teenagers presenting lower prevalence [9, 10]. Although disease is more common in men, the infection rate is equal by sex [3, 10]. The majority of patients with PCM are engaged in farm work [11]. On the other hand, healthy but infected individuals have a variety of occupations, none of which has been demonstrated associated with infection. Environmental conditions predominating in endemic areas are mild temperature, warm and moderately humid weather, abundant watercourses, forests, and acidic soils [3, 12]. Armadillos are the only known animals in which infection, but not disease, has been documented via isolation of the fungus from tissues [13]. Less conclusively, infection has been suggested in other animal species, e.g. horses, cows, and dogs, as determined by positive paracoccidioidin skin tests and/or serological studies [14]. Bats have also been implicated [15]. A recent report mentions the isolation of *P. brasiliensis* from penguin faeces [16].

In spite of the data mentioned above, no studies are available which characterize environmental and occupational variables in both infected and non-infected residents of reservareas. This paper reports factors found significantly associated with *P. brasiliensis* infection in permanent residents of three rural communities in Colombia where children with juvenile PCM had been diagnosed.

METHODS

Location and population

The study was conducted in three rural communities of the province of Antioquia, Colombia, where cases of juvenile PCM in permanent residents had been diagnosed (Fig. 1): (i) Anori (A), characterized by gold mining activities, underground and in rivers; (ii) Campamento (C), an isolated community inaccessible to vehicles, distant from the nearest town (5 h by walking and mule tracks); main exploitation is sugar-cane farming; (iii) Venecia (V), a community located in the middle of the coffee-growing areas. The environmental characteristics of these counties were similar. Altitute varied from 1·325 to 1·700 metres above sea level, the temperature range was 18–24 °C, with means of 20–20 °C, there were high annual precipitations (2·000–3·500 mm^3) and the types of vegetation were those of the very humid to humid pe-mountainous forests.

The cases of juvenile PCM referred as index cases, were diagnosed between 1982 and 1989 and corresponded to three children aged 10–13 years. Study subjects were selected according to the following admission criteria: (i) long-standing residence in the community (defined as a minimum of 2 years of uninterrupted residence by the time the index case was recognized as being ill); (ii) age > 11 years; (iii) no systemic diseases or other viral infections or immunosuppressive therapies at time of the study. Whenever possible those residents who had previous lengthy residence in areas different to the county being surveyed were excluded.

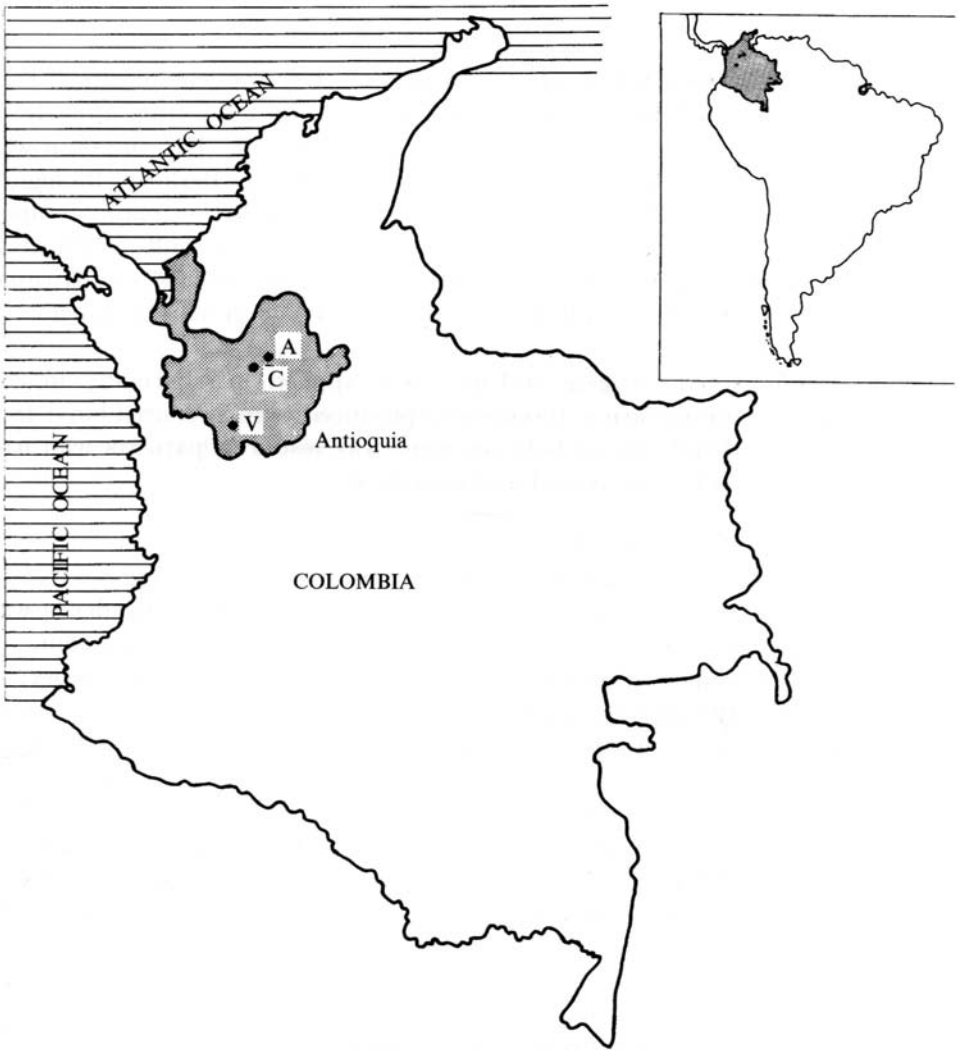

Fig. 1. Location of the communities surveyed (A, Anori; C, Campamento; V, Venecia).

Epidemiological variables

The following data were collected from each of the subjects fulfilling the admission criteria, and from the three index cases: age, sex, and marital status; residence history (defined as places of previous residence, if > 2 months); movements' history (places they visited for < 2 months); activities performed in the community of present residence, including frequency and sites where performed; water sources commonly used for any purpose (cooking, washing, playing, fishing); physical exposure to selected animals (armadillos, bats, local rodents, e.g. *Agouti* spp.). The interview also included questions on past medical history; smoking habits and nutritional status were also determined.

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Skin tests

Each subject was classified as infected or not infected from the results of skin tests with paracoccidioidin. A positive reaction (> 5 mm induration after 48 h) was taken to indicate previous contact with the fungus [17]. Histoplasmin was used as a control of cross-reactivity. This was obtained from Berkeley Biologics, California (Hystolyn CYL) and used as indicated by the manufacturer. Paracoccidioidin was prepared from *P. brasiliensis* mycelia culture filtrates, according to techniques previously published [18]. This antigen was reconstituted at 0·5 mg/ml in phosphate buffered saline (pH 7·4), with 0·04% phenol as preservative.

Freshly reconstituted antigens (0·1 ml) were applied intradermally in the ventral surface of both forearms. Indurations produced were measured blind 48 h post-injection. The reactivity of both antigens was tested in patients who had these mycoses and had been treated and recovered.

Study design and statistical analysis

Dichotomous response variables were used to represent most categories except age which was treated as a continuous variable [19]. Community residence was categorized into three possibilities: (i) single; (ii) more than one (same county); (iii) more than one county. A similar approach was used for movement history, (i) visits restricted to the present county; (ii) visits to neighbouring counties; (iii) visits to distant counties. Nutritional status was graded by age, weight, height, and complexity [20]. Variables universally present in the three communities were also analysed for the total group.

The data were coded and processed using DBaseIII plus software and SPSS software (Statistical Package for the Social Sciences-Version 4, SPSS INC), respectively. Tests for the null hypothesis were χ^2 or Fisher's exact two-tailed test for small numbers.

RESULTS

Characteristics of the population

During the initial visit to the communities 72 permanent residents were identified who fulfilled the admission criteria, 25 in community A, 37 in community C and 10 in community V. However, 18 individuals failed to return for skin tests or interview. Consequently, the study was completed in only 54 permanent residents (A = 18, C = 27, and V = 9). Data not completed from one subject in community A were analysed as missing.

There were no sex differences among the subjects surveyed. Age, however, differed as community C had a younger population compared with A and V (Table 1).

As for residence (Table 2), almost all subjects in community C had lived permanently in that community: on the other hand, half of the individuals in community A, and all those in community V, had lived in places different from their home community. Considering the total group, 63% had always lived in the same community and 80% always in the same county.

Main activities are shown in Table 3. Agriculture was the commonest occupation

Table 1. *Frequency distribution of age*

Age (years)	Community A ($n = 18$) n	%	Community C ($n = 27$) n	%	Community V ($n = 9$) n	%	Total group ($n = 54$) n	%
12–15	3	16·7	8	29·6	2	22·2	13	24
16–25	5	27·8	12	44·4	1	11·1	18	33·3
> 25	10	55·5	7	26	6	66·7	23	42·7

Table 2. *Frequency distribution of community residence*

Community residence	Community A ($n = 18$) n	%	Community C ($n = 27$) n	%	Community V ($n = 9$) n	%	Total group ($n = 54$) n	%
Single	9	50·0	25	92·6	0	0·0	34	63·0
More than one (same county)	6	33·3	2	7·4	1	11·1	9	16·7
More than one county	3	16·7	0	0·0	8	88·9	11	20·3

Table 3. *Frequency distribution of main activities*

Activities	Community A ($n = 17$) n	%	Community C ($n = 27$) n	%	Community V ($n = 9$) n	%	Total group ($n = 54$) n	%
Agriculture (general)	10	58·8	21	77·8	6	66·7	37	69·8
Sugar-cane	1	5·6	19	70·4	0	0·0	20	37·0
Garden farming	6	35·2	2	7·4	2	22·2	10	18·5
Housekeeping	9	50·0	14	51·9	5	55·6	28	51·9
Gold mining	7	41·2	0	0·0	0	0·0	7	13·2
Fishing	7	41·2	8	29·6	2	22·2	17	32·1
Hunting	4	23·5	5	18·5	1	11·1	10	18·9

in all three localities. Sugar-cane farming was almost exclusive to community C, and garden farming was found mainly in community A. Similarly, gold mining was practised only in community A. Housekeeping was the main occupation of half the subjects (women). Fishing and hunting were more common in community A.

Contact with selected animals (Table 4), was relatively common in all communities, especially in A. Smoking was also common; 44·5% in community A, 33% in V and 26% in C, smoked regularly. Altogether, one third of the population smoked.

Undernourishment was prevalent in community C (44·4%), mostly mild to moderate, but rare in the other communities. The two index cases diagnosed were also undernourished. No signs of symptoms suggestive to PCM were recorded.

Skin tests

Ten patients with PCM who had recovered following specific anti-mycotic therapy were tested with both paracoccidioidin and histoplasmin. Nine proved reactive to the former and three to the latter antigen. Five patients who have had histoplasmosis were also skin-tested: positive reactions to histoplasmin but not to paracoccidioidin were obtained in all of them.

Table 4. *Frequency distribution of contact with animals*

Animal	Community A ($n = 17$) n	Community A %	Community C ($n = 27$) n	Community C %	Community V ($n = 9$) n	Community V %	Total group ($n = 54$) n	Total group %
Armadillo	16	94·1	11	40·7	6	66·7	33	62·3
Agouti spp.	12	70·6	9	33·3	1	11·1	22	41·5
Bats	15	88·2	14	51·9	6	66·7	35	66·0
Fish	12	70·6	16	59·3	6	66·7	34	64·2

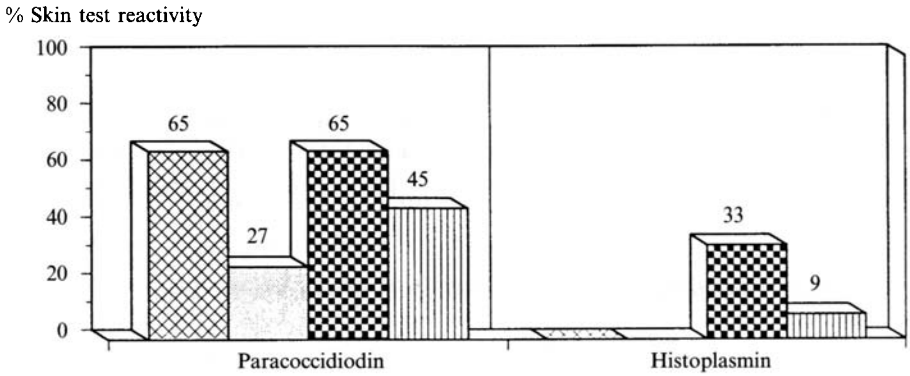

Fig. 2. Results of skin tests reactivity to paracoccidioidin and histoplasmin in community A (diagonal hatch), community C (dotted pattern), community V (check) and total group (vertical bar).

Results of the skin tests are shown in Fig. 2. Considering the total group, 45% had positive paracoccidioidin tests. The reactivity was 65% in communities A and V compared with 27% in community C. Positive histoplasmin reactions were found only in community V, where three subjects were positive; two of these reacted also to paracoccidioidin with large indurations. Paracoccidioidin indurations were larger in community A (mean 18·7 cm) and smaller in community C (mean 12·5 cm). The two index cases recently diagnosed showed anergy to skin tests while the one diagnosed in 1982 had the largest induration registered in this study.

All subjects whose paracoccidioidin test was positive plus the index cases constituted the infected group; all subjects with negative paracoccidioidin tests, formed the non-infected group (controls). In summary, 12/18 (66·7%) permanent residents from community A, 8/27 (29·6%) from community C and 7/9 (77·7%) from community V, were considered infected by *P. brasiliensis*. Altogether, 27/54 (50%) of the total group was considered infected.

Distribution of variables by infection

Age and sex. The percentage of infection in individuals < 15 years age was similar in all communities (Fig. 3). However, while in communities A and V there was a sharp increase in prevalence of infection after 15 years old, with peak figures between 16 and 25 years, infection persisted at low levels in community C. Considering the total group, infected subjects > 25 years of age were more common than younger subjects (12/31 *v.* 15/23, $P = 0·05$). Males were more

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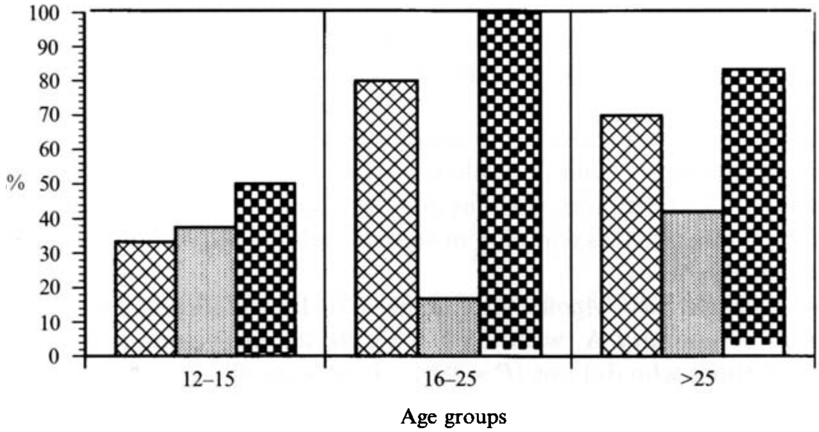

Fig. 3. Distribution of infection by age groups in community A (diagonal hatch), community C (dotted pattern) and community V (check).

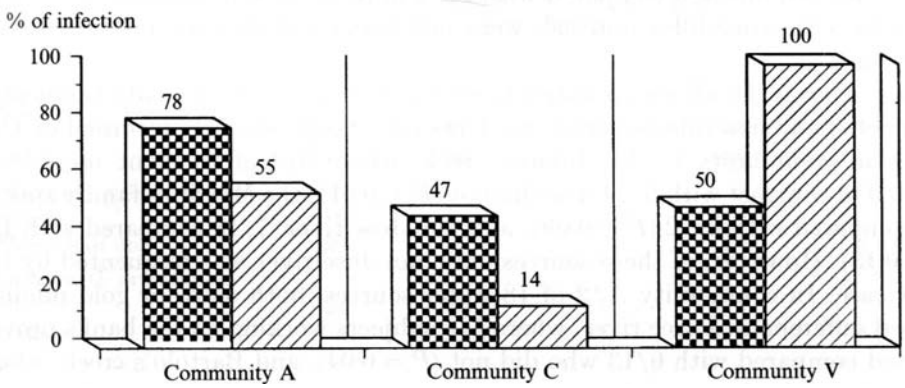

Fig. 4. Distribution of infection by sex in the three communities (male, check; female, diagonal bars).

Table 5. *Infection by community residence*

Community residence	Community A ($n = 17$)		Community C ($n = 27$)		Community V ($n = 9$)	
	n*	%	n	%	n	%
Single	0/5†	0·0	7/25	28·0	—	—
More than one (same county)	8/9†	88·0	1/2	50·0	1/1	100·0
More than one county	3/3	100·0	—	—	6/8	75·0

* Number of infected subjects divided by number of exposed subjects.
† $P < 0{\cdot}01$.

commonly infected in communities A and C, whilst infected females were twice as common in community V (Fig. 4).

Residence and movement history. Table 5 shows the distribution of infection by community residence. None of the whole permanent residents of community A was found infected whilst 8/9 of those who had lived in other communities of the same county were reactive to paracoccidioidin ($P <$ 0·01). Although not as obvious, something similar happened in community C. No differences in infection rates were found when examined by movement history.

Activities. Agriculture was strongly associated with infection in community A ($P < 0{\cdot}01$) but not in the other two. Housekeeping activities were found significantly associated with infection in community V while such activity carried no risk in C (Table 6). Six out of 7 gold miners in community A were found infected compared with 5/10 subjects without gold mining history ($P = 0{\cdot}12$). Infection in community C was twice as common in subjects who fished as in those who did not (4/8 *v*. 4/19, $P = 0{\cdot}13$).

Contact with animals. *Agouti* spp. showed to be significantly associated with infection in community A, with, 83·3% reactivity in persons reporting contact and 20% in those who did not ($P = 0{\cdot}01$). Bats were also significantly associated with infection in this community where 11/15 who had contact were positive compared with 0/2 who did not ($P = 0{\cdot}04$).

This was also true considering the whole group as 60% of subjects who had contact proved infected compared with 28% in those with no contact ($P = 0{\cdot}02$). Contacts with armadillos and fish were not found statistically associated with infection.

Water sources. In all communities most water sources were equally frequented by infected and non-infected subjects; however, exceptions were recorded in 3/14 source in community C: La Juliana creek, where 3/3 of frequent users were infected in contrast with 5/24 who did not ($P < 0{\cdot}01$); the Martinez family spring, 3/5 compared with 6/22 ($P < 0{\cdot}09$); and San José river, 7/18 compared with 1/9 ($P = 0{\cdot}13$). However, of these sources only San José river was frequented by the index case. In community A, 2 of 18 water sources, both used for gold minings showed differences: Porce river, where 5/5 subjects working on the banks proved infected compared with 6/13 who did not ($P = 0{\cdot}04$), and Bartolo's creek, where 3/3 of those who frequented the spot were infected *v*. 8/15 who did not ($P = 0{\cdot}15$). No association with water sources was demonstrated in community V.

Smoking. Considering the whole group, smokers were found significantly more infected than no smokers (13/18 *v*. 14/36, $P = 0{\cdot}03$). This was also true for community A (8/8 *v*. 4/10, $P < 0{\cdot}01$).

Nutrition status. To control the effect of undernourishment on lack of response to skin tests, we determined the nutritional status of the subjects surveyed. When we examined the whole group, we found that the better the nourishment, the more likely it was to obtain a reactive skin test (Mantel–Haenszel test for linear association, $P = 0{\cdot}05$). However, when we examined skin test reactivity controlling by nutritional status in community C (which had the higher undernourishment), reactivity was 26% in normal subjects compared with 33% in undernourished (P = NS).

High v. low infection. To understand the differences observed in the frequency of infection between communities A and V (high) versus C (low), we compared the distribution of variables between these two groups, Statistically significant differences were that subjects in those communities with the higher rates of infection were older, had more complex residence histories and more contact with armadillos than subjects in the community with the lower rates of infection (Table 7).

Table 6. *Distribution of infection by activities*

Activity	Community A ($n = 17$) n*	%	Community C ($n = 27$) n	%	Community V ($n = 9$) n	%	Total group ($n = 54$) n	%
Agriculture (+)	9/10†	90·0	7/21	33·3	4/6	66·6	20/37	54·0
Agriculture (−)	2/7†	28·0	1/6	16·7	3/3	100·0	6/16	37·5
Housekeeping (+)	5/9	55·6	2/12‡	14·3	6/6§	100·0	13/27	48·0
Housekeeping (−)	7/9	77·8	6/13‡	46·2	1/3§	33·3	14/25	56·0

* Number of infected subjects divided by number of exposed subjects.
† $P < 0·01$.
‡ $P = 0·06$.
§ $P < 0·05$.

Table 7. *Variables significantly different in communities with high v. low infection rates*

Variables	High infection (A+V) n	%	Low infection (C) n	%	P value
Age (years)					
12–25	11	37·9	20	74·0	
> 25	18	62·1	7	26·0	< 0·01
Community residence					
Single	9	31·0	25	92·6	
More than one	20	69·0	2	7·4	< 0·01
Contact with armadillos					
Positive	24	85·7	11	40·7	
Negative	4	14·3	16	59·3	< 0·01

Logistic regression analyses. Because different factors associated with infection were found in the total group and in community A, and also because there were differences in communities with high *v.* low infection, stepwise logistic regression analyses were performed to determine how these variables interacted in their association with infection.

In the total group, bats ($P = 0·02$), smoking ($P = 0·03$) and age > 25 years ($P = 0·05$) were found statistically associated with infection. The stepwise analyses showed that after entering bats in step 1, smoking lost its statistical significance ($P = 0·08$), but age did not. When entering age in step 2, smoking lost once more its significance ($P = 0·22$), but bats did not ($P = 0·04$). This shows that among all variables contact with bats was most strongly associated with infection ($P = 0·02$). Age > 25 years came in second place ($P = 0·05$) with smoking no longer having significance.

In community A, variables found significantly associated were the following: residence history outside of actual community ($P < 0·01$), smoking ($P < 0·01$), contact with bats ($P = 0·04$), agriculture ($P < 0·01$), contact with *Agouti* spp. ($P = 0·01$) and use of Porce river ($P = 0·05$). The regression analyses showed that these variables were highly interactive (Table 8). Contact with bats lost its statistical significance after entering community residence, while smoking, agriculture and Porce river gained significance. Smoking, contact with *Agouti* and

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Table 8. *Summary of logistic stepwise analysis for community A*

Step no.	Term*		Log		Improvement		Goodness of fit	
	Ent	Rem	D.F.	Likelihood	χ^2	*P*-Val	χ^2	*P*-Val
0			2	−11·037			22·074	0·024
1	Res		2	−4·499	13·075	0·001	8·999	0·437
2		Res	1	−11·037	13·075	0·001	22·074	0·024
3	Agr		1	−7·439	7·197	0·007	14·877	0·137
4	Bat		1	−3·820	7·238	0·007	7·639	0·571
5		Bat	1	−7·439	7·238	0·007	14·877	0·137
6	Por		1	−5·407	4·064	0·044	10·814	0·289
7		Por	1	−7·439	4·064	0·044	14·877	0·137
8	Smo		1	−6·098	2·682	0·101	12·195	0·203
9		Smo	1	−7439	2·682	0·101	14·877	0·137
10	*Ago*		1	−6·251	2·376	0·123	12·502	0·186

* Ent, entered; Rem, removed; Res, residence; Agr, agriculture; Bat, bats; Por, Porce river; Smo, smoking; *Ago*, *Agouti* sp.

Porce river all lost significance after entering agriculture, while bats importance increased. Entering bats, *Agouti* lost its significance while Porce river re-gained, it; entering smoking, *Agouti* lost significance again. We concluded that residence outside of the actual community and agriculture were the variables with the strongest association with infection in community A.

When considering variables significantly different in communities with high *v.* low infection, the regression analyses revealed that age ($P = 0{\cdot}05$), community residence ($P < 0{\cdot}001$) and contact with armadillos ($P = 0{\cdot}008$) were all significant.

DISCUSSION

This study was conducted to take advantage of the existence of juvenile cases of PCM in permanent residents of three rural communities. For this reason such communities were considered to be true 'reservareas' of *P. brasiliensis*. The poor socio-economical conditions and geographical location of the communities surveyed was associated with reduced population, a fact which increased the likelihood of type II errors during the statistical analyses. Additionally, the reliability of the information collected is expected to be affected by each subject's memory and subjectivity.

The skin tests used to measure infection were shown to be reliable. Paracoccidioidin and histoplasmin proved to be sensitive and specific in preliminary tests. False negative reactions, if occurred, were minimized by exclusion of diseases known to cause anergy and by controlling undernourishment as a cause of anergy [17]. The low number of histoplasmin positive reactions in subjects with positive paracoccidioidin test appear to rule out false positive results due to *Histoplasma capsulatum* infection. In spite of the fact that in Latin America the geographical localization of *H. capsulatum* frequently overlaps that of *P. brasiliensis* [1], this was not the case in communities A and C, the only areas where histoplasmin reactions were detected.

The rates of paracoccidioidin positivity found in communities A and V (> 65%) are among the highest reported for a particular population [9, 11]. Even the lower rate round in community C as well as the average for the total group, are higher than those previously reported for different endemic areas in Colombia [10].

The size of the induration produced by paracoccidioidin differed according to the county and was larger in community A and smaller in community C. This suggests that subjects from community A had been exposed to a greater antigenic challenge than those in C.

The relationship between *P. brasiliensis* and its environment has been elusive [3]. This has not been the case for the other systemic mycoses. Since the first isolation of *H. capsulatum* from soil by Emmons in 1949, it has been shown that bird droppings and bat faeces provide the optimal environment for *H. capsulatum* growth and persistence [21]. It has been also demonstrated that the prevalence of histoplasmin skin reactivity in children correlates with the number of bird roosts nearby. Vigorous clean up of accumulated bird droppings has been shown to be associated with local outbreaks of histoplasmosis [21]. However, in many instances neither the source of *H. capsulatum* nor the human activities causing this outbreak have been determined [22].

Blastomyces dermatitidis was isolated from wet earth containing animal droppings in association with a local outbreak [23]. The fungus microfoci was also noted to be associated with bodies of water [24]. However, many other investigations done in locations where common-source exposure was suspected proved unsuccessful in recovering the organism from soil [25].

In the case of *P. brasiliensis*, lack of outbreaks and the prolonged latency period of the infection have hindered determination of the habitat [3]. This exploratory case control study allowed detection of various factors significantly associated with infection. In the case of community A, we found that 90% of the infected subjects had previously resided in other communities of the same county, although not the same for everyone. For this reason a single point-source of infection in this community seems unlikely. However, the majority of infected subjects had lived in communities surrounding Porce river, which by itself, was found significantly associated with infection as a water source. We may postulate that Porce river or its banks are the body of water supporting the *P. brasiliensis* microniche [3], responsible for the infections recorded in the community. In support of this hypothesis is the finding that the only place where index case from community A lived before the onset of disease was the hillsides of Porce river. All members of his family living with him at that point were also found infected. Gold mining, mainly in Porce river, was common in infected subjects, suggesting that this may be one of the activities leading to contact with the fungus. Agriculture, mainly related to vegetable gardening, was significantly associated with infection in this community. The fungus may benefit from the nitrogen-rich and humid soils characteristic of vegetable and fruit gardens, with gardening providing the opportunity for contact. The significant association of contact between bats and *P. brasiliensis* infection in this community also deserves discussion. Although such contact may be explained, partially at least, by residence history and agriculture it is of interest that contact with bats was the variable most strongly associated with infection when considering the total group. This raises the old controversy about the role of bats in the ecology of *P. brasiliensis* [15, 26].

Community C, although geographically related to Anori, showed completely different skin tests results. However, community C had the advantage that almost all subjects tested were life-long residents.. The 8 infected subjects should have contacted *P. brasiliensis* microniche somewhere around that community. Infection was not associated with a particular activity or contact with animals. Furthermore, although parents, brothers, and sisters of the index case were tested, no one was found infected. Only the frequent use of one water source was statistically associated with infection. Yet, not all infected subjects frequented this point and neither did the index case. Nonetheless, all infected individuals and the index case had in common fishing in San José river, a water source also frequented by non-infected subjects. It appears that in this community the microniche probably lies near water sources not accessible to all residents.

Community V occupies a different geographical niche. Most of the subjects had lived before in other counties, so there is no way to assure that the infection was acquired in such a community, as was the case for the majority of the infected subjects in communities A and C. Although the low number of permanent residents found here makes the analyses difficult, it is interesting that housekeeping activities were statistically associated with infection.

The finding of a higher infection rate in subjects > 25 years is in agreement with previous studies showing that infection is less common in younger people, probably because they have had less opportunities to contact *P. brasiliensis* [9–11]. Additionally, permanent residence in a community may hinder the inhabitants from reaching other more appropriate fungal micro-niches. It is also interesting that contact with armadillos, the only animals in which *P. brasiliensis* infection has been documented, was significantly more common in communities with higher infection rates. We can hypothesize that the niches for armadillos and *P. brasiliensis* are environmentally related.

This case-control study proved useful to explore factors associated with infection in each community surveyed. The results may orient future studies aimed at the isolation of this human pathogen from the environment, an essential step for full characterization of the fungus' natural habit.

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