Climate and acute/subacute paracoccidioidomycosis in a hyper-endemic area in Brazil

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Background Paracoccidioidomycosis (PCM) is Latin America’s most prevalent systemic mycosis, carrying an important social burden. Its agent, Paracoccidioides brasiliensis, has rarely been identified in nature. Studies characterizing acute/subacute PCM incidence and their relationship with climate variables are not available. This work analysed a series of acute/subacute cases that occurred in the Botucatu area, São Paulo State, Brazil, from 1969 to 1999, as an outcome of weather variability.

Methods Stepwise regression of annual data was applied to model incidence, calculated based on 91 cases, from lagged variables: antecedent precipitation, air temperature, soil water storage, absolute and relative air humidity, and Southern Oscillation Index (SOI).

Results Multiple regression analyses resulted in a model, which explains 49% of the incidence variance, taking into account the absolute air humidity in the year of exposure, soil water storage and SOI of the previous 2 years.

Conclusions The correlations may reflect enhanced fungal growth after increase in soil water storage in the longer term and greater spore release with increase in absolute air humidity in the short term.

Keywords Paracoccidioidomycosis, climate, environment

Introduction Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the dimorphic fungus Paracoccidioides brasiliensis. It is endemic in Latin America, with cases being reported in almost every country, but especially in Brazil.1 If not diagnosed and adequately treated, it can lead to serious and lethal disseminated forms, with progressive involvement of lungs, tegument, adrenals, central nervous system and the mononuclear–phagocytic system. It usually requires long periods of treatment during which the patients remain out of their productive activities. Since PCM predominantly affects male agricultural workers at their most productive ages, it has an important social impact,2 being the seventh leading cause of death among all chronic infectious disease
in Brazil. Currently there are no recommended measures to protect against infection.

PCM has two major clinical presentations. The most common is the chronic (adult) form, which affects mainly patients over 30 years old and has a long latency period (years to decades) and the other more infrequent form is the acute/subacute (juvenile) form (AF), which is usually more severe and develops more rapidly, affecting mainly children, adolescents and young adults. The incubation time is not precisely known, but is considered to range from weeks to several months. Due to the fact that children and young adults have a restricted migratory profile, they are considered epidemiologic sentinels. As the place where they live is likely the same where they have acquired the infection, they may provide more trustworthy data concerning the habitat of *P. brasiliensis*.

Infection is acquired through inhalation of airborne propagules. However, even in endemic countries, the geographical distribution of the disease is heterogeneous and appears associated with specific environmental conditions such as mild temperatures, fertile soils and high humidity. Many studies have focused on defining the niche of the fungus based on the location of the patient’s residence. However, since acute/subacute cases present shorter incubation periods, they may allow more accurate spatial and temporal analyses.

To date, few variations in incidence have been observed. Besides land use changes, which causes soil disturbance and the exposure of larger numbers of individuals, *P. brasiliensis*, like other human pathogenic dimorphic fungi, responds to moisture content and temperature, therefore a relationship may exist between climate conditions and PCM in its AF. However, a climate-based explanation for the space–time pattern of acute/subacute PCM variability has never been investigated.

The aim of this work was to verify the hypothesis concerning climate influences on acute/subacute PCM incidence. For this purpose, we analysed a series of acute/subacute cases diagnosed in a hyper-endemic area, Botucatu and the surrounding region, São Paulo State, Brazil, and reflected them against climate variables during the period 1969–99.

**Methods**

**Acute/subacute PCM data, demographic data and study area**

The Committee for Ethical Research of the Medical School of the São Paulo State University approved this study. Informed consent was not necessary because the study was retrospective and no personal identifiers were used.

Acute/subacute PCM incidence was explored in relation to weather variables through ordinary least-squares regression analysis. The analyses were performed in the R software, version 2.6.1. Medical records of PCM patients from the University Hospital (UH-UNESP), from January 1969 to December 1999, were reviewed. Patients included in this study had the diagnosis of acute/subacute PCM confirmed by the identification of the agent in clinical specimens. Year of diagnosis, age, gender, municipality of residence and clinical forms of the disease were obtained from patients’ medical records. The annual incidence rates were calculated based on population data by municipality by year from the São Paulo State System for Data Analysis Foundation (SEADE). The study area was defined as comprising 44 municipalities between 48° and 48°30’ WGr. and between 22°12’ and 23°42’S. It should be stated that all patients with suspected PCM within the study area are necessarily referred to the UH. The UH is the single reference hospital in this area, is a free-access, public hospital that has a mycologic diagnostic sector.

**Environmental variables**

**Precipitation**

Daily precipitation data from 37 rain gauges corresponding to the study area, from 1966 to 1999, were obtained from the Water and Electrical Energy Department (DAEE) of the State of São Paulo. Unfortunately, many rain gauges were closed after 1999, which limited the study period up to this year.

**Air temperature, absolute and relative humidity**

Absolute air humidity is the amount of water vapour contained in a particular volume of air. Relative air humidity represents the ratio between absolute humidity and the humidity needed for air parcel saturation. Air temperature, absolute and relative humidity at the level of 1000 hPa were obtained from National Center for Environmental Prediction/National Center for Atmospheric Research (NCEP/NCAR) reanalysis with spatial resolution of 2.5°. Air temperature data corresponded to overall mean, since mean maximum and minimum temperatures were not available at the NCEP/NCAR reanalysis. The reanalysis dataset was compiled from different observational sources (e.g. surface networks, ships and satellites) adjusted by an atmospheric model, and is commonly used as actual data in studies of atmospheric simulation. Since this dataset has low spatial resolution, the average over 22°30’ and 25°S, and 48°30’ and 50°W, was considered to represent the overall area.

**Soil water storage**

The sequential water balances for the 37 rain gauges were calculated following the method of Thornwaite and Mather. This method considers variation in soil water storage as an exponential function, which involves available water capacity in soil and loss of accumulated water. Evapotranspiration was computed based on monthly mean air temperature for the study.
area for the period from NCEP/NCAR reanalysis and latitude of each rain gauge. Values of available water capacity in soil for each rain gauge corresponded to 75, 100 and 125 for soils classified according to their texture as: up to 15% of clay particles, from 15% to 35 and >35%, respectively. Precipitation for computing the soil water storage corresponded to monthly precipitation from data of DAEE for each rain gauge for the period.

Southern Oscillation Index
Data of Southern Oscillation Index (SOI) were obtained at the National Oceanic and Atmospheric Administration website. This index is the normalized surface air pressure anomalies difference between the island of Tahiti and Darwin, Australia. A SOI of two standard deviations below the climatologic mean corresponds to a rise in sea surface temperature at central-east Equatorial Pacific basin and is known as El Niño. The advantage of this index is that it summarizes the complex climate space and time variability, thus permitting the assessment of the interaction among the climatic variables in modelling studies.

Averages of the environmental variables for the entire study area were calculated.

Modelling
Linear regression modelled acute/subacute PCM incidence considering as independent variables annual data from up to 3-year lagged, starting in 1966: antecedent precipitation, air temperature, soil water storage, absolute and relative air humidity and SOI. All variable data were linearly detrended, since two data series can trend together because of other unobserved factors. Even if those factors are unobserved, it is possible to control them by directly controlling for the trend. It was assumed that antecedent environmental conditions are very important for fungal growth based on other dimorphic pathogenic fungi and conidial liberation. Data were available and analysed in annual timescale. Consequently, cases that occurred in 1 year were likely not associated with the mean environmental conditions of that concurrent year. In addition, a period of 11 months between the moment of infection and date of diagnosis was assumed. This period takes into account the incubation time and the time lag that the patient, once symptomatic, needed to search a healthcare unit and was provided with the results of the diagnostic tests. Thus, it is conceivable that it took at least 1-year lag for the environmental variables.

Findings at $P < 0.05$ were considered significant.

Results
From 1969 to 1999, 783 cases with confirmed diagnosis of PCM were recorded. Among these, 180 were classified as presenting the AF and 91 (50.6%) of the patients resided within the study area.

Incidence rates of acute/subacute PCM in the study area can be seen in Figure 1. Atypical incidences have occurred in 1985 (1.24/100 000 inhabitants) and 1983 (1.11/100 000 inhabitants). Exploratory bivariate analyses showed that the incidence was positive and linearly correlated with air temperature ($r = 0.45$, $P < 0.05$) and with absolute air humidity ($r = 0.50$, $P < 0.01$) in the year preceding diagnosis (Figure 1a and b). Other variables that were tested were precipitation, relative humidity, water soil storage and SOI, which gave non-significant correlations (Table 1).

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Backward and forward stepwise analyses aimed to select the most important variables that could explain the acute/subacute PCM incidence. Two models were obtained and evaluated. The first model explained 31% ($P < 0.002$) of the incidence variability in this period, taking into account the 1-year lagged absolute air humidity and 3-years lagged soil water storage (Figure 2a, Table 2). In the second model, adding the 3-years lagged SOI, the model explained 49% ($P < 0.0001$) of the incidence variability (Figure 2b and Table 2). The models were assessed in relation to the linear model assumptions using the global test and the assumptions were acceptable, at a level of
Table 1 Correlations between acute PCM incidence and lagged variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>soi1</td>
<td>-0.20</td>
<td>0.28</td>
</tr>
<tr>
<td>temp1</td>
<td>0.45</td>
<td>0.011</td>
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<tr>
<td>rh1</td>
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<td>0.22</td>
</tr>
<tr>
<td>abs-hum1</td>
<td>0.50</td>
<td>0.005</td>
</tr>
<tr>
<td>pcp1</td>
<td>-0.03</td>
<td>0.89</td>
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<tr>
<td>sws1</td>
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</tr>
<tr>
<td>soi2</td>
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</tr>
<tr>
<td>temp2</td>
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</tr>
<tr>
<td>rh2</td>
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<td>0.32</td>
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<tr>
<td>abs-hum2</td>
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<td>pcp2</td>
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<tr>
<td>sws2</td>
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<td>0.10</td>
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<tr>
<td>temp3</td>
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<tr>
<td>rh3</td>
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</tr>
<tr>
<td>abs-hum3</td>
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<td>0.24</td>
</tr>
<tr>
<td>pcp3</td>
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</tr>
<tr>
<td>sws3</td>
<td>0.25</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Correlations with climatic variables.
soi = Southern Oscillation Index; temp = overall mean temperature; rh = relative humidity, abs-hum, absolute humidity; pcp = mean annual precipitation; sws = soil water storage; the numbers 1, 2 and 3 after the variables denotes the variable occurring 1 year earlier of the incidence and so on.

Table 2 Adjusted $R^2$, standardized ($\beta$) coefficients and significance for the regression models explaining acute PCM incidence, 1969–99

<table>
<thead>
<tr>
<th>Model</th>
<th>Adjusted $R^2$</th>
<th>Coefficient</th>
<th>P-value</th>
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</thead>
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<td>Model 1</td>
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<td>0.002</td>
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<td>abs_hum1</td>
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<tr>
<td></td>
<td></td>
<td>sws3</td>
<td>-0.0004</td>
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<tr>
<td>Model 2</td>
<td>0.49</td>
<td>-6.2331</td>
<td>0.0000</td>
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<td></td>
<td></td>
<td>abs_hum1</td>
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<td></td>
<td></td>
<td>sws3</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>soi3</td>
<td>-0.1864</td>
</tr>
</tbody>
</table>

Models of incidence on the basis of climate conditions.
abs-hum1 = absolute humidity occurring 1 year earlier of the incidence; soi3 = Southern Oscillation Index occurring 3 years earlier of the incidence; sws3 = soil water storage occurring 3 years earlier of the incidence.

Discussion

Variation in the incidence of deep mycoses has been claimed to be linked to atmospheric variables in some studies, coccidioidomycosis being the best-studied example. The influence of climate on
coccidioidomycosis has been studied since 1957, but only more recently some good models that include atmospheric variables have been developed to predict its incidence in endemic areas such as California and Arizona. However, some studies have questioned the importance of the climatic variables in the incidence of these mycoses. Compared with other dimorphic, soil-dwelling, human pathogenic fungi of the Onygenales order, such as Blastomyces dermatitidis, Coccidioides immitis, Coccidioides posadasii and Histoplasma capsulatum, P. brasiliensis is the less comprehended in its ecology. Moreover, no outbreaks have yet been reported in PCM making the characterization of its ecological niche even more difficult. All of the above mentioned pathogens typically present some common features: restricted (or localized) geographic distribution and ability to grow as saprobes in soil-related materials in the environment. Links between fluctuations in PCM incidence and climate could seem unlikely because most cases of PCM are of adult form and the latency period is unpredictably long. In fact, among the many limitations in making studies based on incidence is the noise due to uncertainty in obtaining the precise date of infection/exposure. In general, there is considerable variability in incubation time and/or time elapsed until the patient with mycoses such as Blastomycosis, coccidioidomycosis and PCM seeks medical attention. This variability may cause noise when using a monthly scale to analyse the data. In this regard, PCM has the advantage that the two forms of the disease are easily distinguishable clinically. The present analysis of acute/subacute cases demonstrates that climatic connection may exist despite this noise. However, when combined with the use of annual incidence this type of uncertainty may be minimized. If, on one hand, the use of incidence data adds some uncertainties, on the other, this series of acute/subacute PCM incidence is currently the only ‘measure’ of the fungus temporal variability in the environment. Animal sentinels as the armadillo Dasypus novemcinctus and molecular techniques have important limitations because sampling wild mammals, besides being time and cost consuming, is only parsimoniously authorized by environmental protection agencies, and molecular techniques do not discriminate well between dead and viable cells. Seasonal analysis of fungal counts in soil, as suggested for Coccidioides spp, are not feasible for P. brasiliensis due to the very poor isolation rates of the fungus from such samples. This first approach at explaining acute/subacute PCM incidence based on climate variability suggests some relevant implications to the biology of P. brasiliensis. Greater absolute humidity is found to be significant in the year before incidence and, according to our estimates, in the probable year of infection. Although laboratory studies have not been carried out to observe the influence of humidity on P. brasiliensis conidial liberation, a parallelism can be made with two of the closest pathogenic fungi (B. dermatitidis and H. capsulatum). Laboratory studies with these fungi have demonstrated that the conidia remains attached to conidiophores that were violently shaken by air currents in an observation chamber, whereas >99% of the conidia were readily released when the conidiophores were first wetted. Consistent with this, it has been hypothesized that rain and/or the mist and fog commonly found along the banks of waterways can lead to the release and dissemination of B. dermatitidis conidia. In the case of P. brasiliensis, an exploratory case control study carried out in Colombia postulated that the riverbanks were the body of water supporting the fungus microniche. Also armadillos infected with P. brasiliensis have been detected mainly in anthropic disturbed riparian areas, near water bodies.

In addition, higher soil water storage 2 years before exposure may explain the already suggested relationship between P. brasiliensis and rainfall/humidity. The results obtained here suggest that there is fungal growth after increase in soil water storage in the longer term, as has been noted for Coccidioides spp, and probably greater spore release with increase in absolute air humidity in the short term. When modelling coccidioidomycosis incidence using seasonal data, Comrie found out that the most striking result was the dominant role of precipitation during the normally arid foresummer 1.5–2 years before the season of exposure and concurrent seasonal dust and precipitation. Results presented here corroborate the climate influence within the same lag time.

It is worth noting that the connection between acute/subacute PCM incidence and water budget reinforces the importance of the complex environmental system as a whole and not only the precipitation. It can be hypothesized that an antecedent period of high soil moisture is necessary for the filamentous form of the fungus to establish itself in the soil at a sufficient high density to allow production of high amounts of propa- gules, hence increasing the likelihood of a ‘productive’ human infection. There is indirect evidence that P. brasiliensis grows preferentially below the soil...
surface, perhaps at 2–20 cm from the surface, as has been postulated for *Coccidioides* spp., or even deeper when considering the high rate of armadillo infection that occurs in endemic areas. Human activity then dislodges the superficial portion of the soil, exposing the filamentous, spore producing form of the fungus. If the absolute humidity is high enough at that moment, the spores are wet and then freed and aerosolized. Fungal growth in deeper layers, instead of at the soil surface, would be advantageous because, as for other pathogenic fungi, *P. brasiliensis* grows slowly and is likely bypassed by its competitors abundantly present in the first centimetres of the soil layer (A horizon). The high frequency of persons living in endemic areas with evidence of previous or ongoing infection who do not develop the disease may well represent occasions when the person is exposed to insufficient amounts of spores to override the host’s immune response, resulting in an ‘abortive’ infection.

Restrepo suggested a possible correlation between PCM cases and annual precipitation in the year preceding diagnosis in Colombia, from 1979 to 1984. In our study, only the 3-year lag precipitation presented a weak, non-significant correlation with incidence. However, SOI better explains acute/subacute incidence than precipitation. ENSO is the most important coupled ocean–atmosphere phenomenon to cause global climate variability on inter-annual timescales. In general, ENSO events are accompanied by changes in cloud amounts, trade winds and rainfall. Then, SOI reflects the interaction whereas precipitation is only one component of the complex climatic system. According to Stenseth *et al.*, in many situations, the most important climate effects on individuals result from weather variables interaction. In such cases, a large-scale climatic index may be a better representation of climatic effects than any single local weather variable. Good correlations with SOI have been observed for vector-borne diseases such as malaria, with short lags (2–3 months) between SOI and incidence. For PCM and coccidioidomycosis, a longer lag is necessary for changes in environmental conditions to affect incidence.

This study shows that even an endemic systemic mycosis like PCM responds to climatic variability. The disease incidence is not only driven by climate, but it seems real enough that climate plays an important role in the AF of this mycosis. Even though the number of cases along the 31-year period yields annual rates whose variation might be strongly affected even by a single occurrence in a given year, the model achieved good fitting and provided significant estimates of effect of absolute air humidity, soil water storage and SOI. Modelling can also be useful taking into account the global climatic changes. Local weather patterns may change following the greenhouse effect reinforcement; actually, precipitation can be intensified in specific months. In this case, predictions of PCM incidence variation could be made. In addition, this study can provide better opportunities to determine the local environmental variables that likely influence the biology of the fungus, namely, its survival in soil, its competitive advantages, its capacity to produce conidia and the conditions that favour enhanced infection of humans. Another approach, the ecologic niche modelling, has been used to assess the geographic and ecologic distribution of pathogenic fungi with interesting results. Both approaches would render easier the task of isolating the fungus from nature and defining its ecological niche.

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**Conflict of interest:** None declared.

**KEY MESSAGES**

- Our study provides strong evidence that an endemic systemic mycosis, PCM responds to climatic variability.
- The best linear regression model provided significant estimates of effect of absolute air humidity, soil water storage and SOI on the incidence of the mycosis.
- The results suggest that there is fungal growth after increase in soil water storage in the longer term (~2 years), and probably greater spore release with increase in absolute air humidity in the short term (~1 year).
- This modelling can provide better opportunities to determine the local environmental variables that likely influence the biology of the fungus and may help future studies aimed at defining the ecological niche of *P. brasiliensis*. 
References


35. Fisher FS, Bultman MW, Johnsen SM et al. Coccidioides niches and habitat parameters in the Southwestern
Commentary: Environmental determinants of dimorphic systemic mycoses—the macro and the micro

Dennis J Baumgardner¹²

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The article by Barrozo et al.¹ in this issue is a most interesting investigation of the effect of climatic features on the yearly incidence of paracoccidioidomycosis (PCM), a serious systemic mycosis of Latin America. The epidemiology of PCM is very difficult to study due to the long and unpredictable latency period of the common (adult) form of the disease. The authors have taken care to restrict their analysis to area residents with the uncommon acute form of the disease. The authors discuss the limitations of their study, including the particular concern of the small number of cases (91 over 30 years) and presumption of comprehensiveness of the cases. A small number of missed cases or any number of unmeasured factors or change in the distribution of cases within the study area during that time may have significantly altered the results.

Nonetheless, this work provides further evidence that climatic and human activities on the ‘macro’ level act in concert with ‘micro’ ecological factors, perhaps in association with mammals, to effect growth, dissemination and ultimate human infection by the dimorphic systemic fungi. These organisms grow as a mould in the environment, sporulate and infect humans. At body temperature they convert to the yeast form. Their micro-ecological niches are still largely undefined, in part because of the difficulty in isolating the respective organisms from the environment (with the possible exception of Histoplasma).

For PCM, the authors¹ postulate that soil water storage and El Niño activity 3 years before, and absolute air humidity the year before the case incidence explains 49% of the yearly variance. Paracoccidioides brasiliensis has a strong association with armadillos, likely beyond being merely sentinel animals. Positive armadillo-isolated areas from this region in Brazil were typified by nearby water sources and disturbed vegetation, temperatures 15–26°C and shaded sandy or clay soils of variable fertility at pH 3.9–6.0.² Coccidioidomycosis, perhaps the best-studied dimorphic fungus regarding environmental determinants, is endemic to southwestern North America and parts of Central and South America. Ecological niche models match well with epidemiologic data.³ Regression-based climate modelling has had variable success predicting the future case incidence, perhaps due to human factors in various locales; however, climate accounts for much variability in southern Arizona. Here, increased antecedent precipitation, then increased temperatures and drought, followed by wind or excavation of dust dispersal leads to

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Reference