

An autochthonous case of paracoccidioidomycosis in a new area of Ceara State, Northeastern Brazil

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ABSTRACT

The largest endemic areas of paracoccidioidomycosis (PCM) in Brazil comprise the humid agricultural regions of the Southeast, South, and, recently, the Midwest and North regions. The Ceara State, located in the Brazilian Northeast region, presents semi-arid climate in most of its territory, characterized by high temperatures, scarce vegetation and low humidity. The objective of the present study was to describe a new autochthonous case of paracoccidioidomycosis from a distinct area of Ceara and review the characteristics of PCM occurrence in Northeastern Brazil. The patient was a 65-year-old male farmer who denied traveling outside the Ceara State or living in other locations. He was born and lived in the rural area known as Camara, bordering the municipalities of Itapaje and Itapipoca. Camara is one of the highest areas (around 720 m of altitude) of the Uruburetama mountains that exhibits tropical forests and is located in Northern Ceara, distant 139 km from the capital, Fortaleza. The patient sought for care, complaining of an oral lesion that appeared over the past three years. The hard palate lesion biopsy revealed multinucleated cells with cytoplasmic inclusions, compatible with PCM. After culture, *P. brasiliensis* was identified by polymerase chain reaction. Serological testing for PCM was reagent. The patient was treated with itraconazole for approximately 17 months, persisting free of symptoms after 15 months of follow-up. Regarding this new autochthonous case in the Ceara State, PCM should be considered in the differential diagnosis of patients with suggestive clinical manifestations, proceeding from the mountainous areas of Ceara.

KEYWORDS: Fungal diseases. Paracoccidioidomycosis. Ceara. Northeastern Brazil. *P. brasiliensis*

INTRODUCTION

Paracoccidioidomycosis (PCM) is an endemic systemic mycosis that is highly prevalent in South America. It is caused by the dimorphic fungi *Paracoccidioides* spp., and is distributed in at least 5 species: *Paracoccidioides brasiliensis* (former S1 phylogenetic group), *Paracoccidioides americana* (former PS2 phylogenetic group), *Paracoccidioides restrepiensis* (former PS3 phylogenetic group), *Paracoccidioides venezuelensis* (former PS4 phylogenetic group) and *Paracoccidioides lutzii* (former Pb01-like phylogenetic group). The species *P. lutzii* was identified in restricted endemic areas such as the Brazilian Midwest and North regions¹.

In Brazil, the largest endemic area includes the Southeast, Midwest and South regions. However, in the 1990s, along with the growth of new agricultural areas, the disease spread to the Eastern border of the Amazon River and, subsequently, to the Western Amazon region, with an incidence of 9.4 cases per 100,000 inhabitants^{2,3}.

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Due to geographic characteristics, the Brazilian Northeast region, which, in its majority exhibits semi-arid climate, has shown low endemicity².

A previous study reported only two autochthonous cases from Palmacia in Ceara State⁴. The objective of the present study was to describe a new autochthonous case from a distinct area of Ceara State and review the characteristics of PCM occurrence in Northeastern Brazil.

CASE REPORT

The patient was a 65-year-old male farmer that worked primarily on corn and bean crops but also raised a few dairy cows. He was born and lived in Camara, a rural area of the Uruburetama mountains, and denied traveling outside Ceara State or living in other locations, nor did he come in contact with armadillos.

The patient sought for care on September 8, 2016 at the outpatient clinic of Systemic Mycoses of the Sao Jose Hospital of Infectious Diseases, in Fortaleza, Ceara State, complaining of an oral inflammation that had been upsetting him during the past three years. Fever or respiratory symptoms were not reported. The infiltrative oral lesion was located on the palate (Figure 1). In addition, an ulcerative lesion was found on the skin and right nasal mucosa (Figure 2). Hypophonic cardiac sounds and reduced vesicular murmurs in both lungs were also found. The abdominal examination was normal, and cervical lymph node enlargement was not observed. The patient reported cigarette consumption of 40 packs/year for 40 years but had stopped smoking that past year. After undergoing a chest X-ray, nodules with predominantly



Figure 1 - Infiltrative oral lesion, painful and smelly, similar to moriform stomatitis, with an irregular hyperemic surface located on the palate.



Figure 2 - Ulcerative lesion in the right nasal mucosa and skin.

central distribution were observed affecting both lungs, some showing signs of cavitation and bilateral perihilar reticular infiltrate (Figure 3).



Figure 3 - Chest x-ray showing nodules with predominantly central distribution affecting both lungs, some with signs of cavitation and bilateral perihilar reticular infiltrate.

A previous biopsy of the hard palate lesion revealed a squamous pattern of mucosal fragments presenting granulation tissue associated with chronic lymphohistiocytic infiltrates and multinucleated cells, with cytoplasmic inclusions compatible with a fungal infection, evidenced by PAS and Grocott stains, suggestive of *Paracoccidioides* spp. yeast forms. Computed tomography (CT) scanning of the face

revealed a maxillo-ethmoidal sinusopathy, left-deviated bone septum, palatine bulging to the right and an irregularity of the cortical bone of the hard palate on the left side. An imprint of the palatal lesion showed a moderate presence of intra- and extracellular yeast structures with considerably variable sizes (small and oval, medium, large and rounded) by Giemsa staining; *Leishmania* was not found in the analyzed material.

A new biopsy was performed for microbiological examination and treatment with itraconazole (200 mg daily) was initiated at the same day the patient was first seen at Sao Jose Hospital. *P. brasiliensis* was identified by the polymerase chain reaction (PCR) after fungi growth in culture. For species identification, DNA extraction of yeast cells was performed, based on a previous protocol⁵ with minor modifications, using the DNeasy Plant Mini Kit (Qiagen™, USA). The obtained product was subjected to PCR using panfungal primers ITS1 (5'-TCCGTAGGTGAACCTG CCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The final 25 µL of PCR mix was composed of 2.95 µL of ultrapure water, 5.0 µL of buffer (Promega, Madison, Wisconsin, USA), 5.0 µL of MgCl₂ (Promega, Madison, Wisconsin, USA), 5.0 µL of dNTP (Promega, Madison, Wisconsin, USA), 2.5 µL of each primer (Invitrogen, Thermo Scientific, Waltham, Massachusetts, USA), 0.05 µL of Go Hot Start Taq polymerase (Promega, Madison, Wisconsin, USA), and 2.0 µL of DNA template. PCR conditions were: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 20 s, annealing at 56 °C for 15 s, and extension at 72 °C for 65 s, and a final extension step at 72 °C for 5 min. The presence of PCR products (639 bp) was verified in 1% agarose gel electrophoresis. Then, amplicons were sequenced and compared with the *P. brasiliensis* CBS 372.73 strain (GenBank accession MH860706.1) using the Basic Local Alignment Search Tool (Nucleotide Blast™), resulting in the identification of *P. brasiliensis* with 98.8% of similarity.

Double radial immunodiffusion for PCM was positive. The patient was treated with itraconazole for 17 months and evolved with lesion improvement. A white scar over his hard palate caused a retraction in the tonsil topography, but there was no clinical disturbance. The individual had a normal chest X-ray at the end of treatment and had also undergone a new CT scan of the face, which showed a sinusopathy and left-deviated bone septum, but did not evidence the previous bone alterations in the palatal region. He was followed-up until June 6, 2019, persisting free of symptoms.

DISCUSSION

The geographical distribution of *Paracoccidioides* spp. is predominantly limited to certain regions of North, Central and South America, characterized by high humid climates

and mild temperatures. However, the present study reports an autochthonous case of PCM in a new mountainous area of the Ceara State, a territory that majority lays in the semi-arid region, characterized by high temperatures, scarce vegetation and low humidity, and mean annual rainfalls of 800 mm per year.

The Camara region (residence of the patient) is located on the borders of the Itapaje and Itapipoca municipalities, in the center of the Uruburetama mountains (Figure 4), Northwestern Ceara State, at -3.63333 (3° 37' 60" South) latitude and -39.6333 (39° 37' 60" West) longitude. The area of Camara is situated at around 720 m of altitude and its vegetation is also composed of rainforest. The Uruburetama mountains present significant rainfall indexes varying from 900-1,300 mm, between January and June, and among the observed agricultural activities are banana cultivation and corn and bean crops⁶.

The previous two autochthonous cases were reported in residents of Palmacia (Figure 4), located in the mountains of Baturite, 74 km West of the capital of Ceara⁴. Palmacia is a predominantly rural city, located at 4° 09' 01" South latitude and 38° 50' 47" West longitude, at an altitude of 425.11 m. It presents tropical hot-humid climate, with average annual rainfall of 1,386.6 mm, mean annual temperatures between 24 °C and 26 °C⁷, and its economy is based primarily on agriculture, namely banana production^{4,7}.

Approximately 80% of the PCM cases reported worldwide were from Brazil, mainly in the Southeast, Midwest and South regions². A second endemic area is located along the Eastern border of the Amazon region⁸, and a third includes the Western Amazon region³. Alterations in the epidemiology of PCM have been observed in Brazil, due to factors such as: a) the migration of agriculture and livestock activities to unexplored territories; b) change to more protective manners, such as mechanical plantation in older endemic areas c) manual family subsistence farming in the Northeast, exposing farm workers to inhalation of fungal spores from the soil. Other implicated factors include increased population density, climate changes, deforestation and soil movement, factors that have caused an increase in PCM cases in endemic areas, mainly of acute/subacute forms^{2,9}.

The prevalence of PCM found in the Northeastern region by paracoccidioidin intradermal testing was of 24.2% in Piauí, 32.1% in Ceara, 11.2% to 20.4% in Rio Grande do Norte, 19.5% to 57.4% in Paraíba, 11.2% in Alagoas and 5.6% to 21.7% in Bahia¹⁰. High prevalences have been observed in endemic areas, including Southeastern Brazil (39%) and Colombia (45%)².

In Pereiro city, located in a mountainous area distant 342 km from Fortaleza, the prevalence of

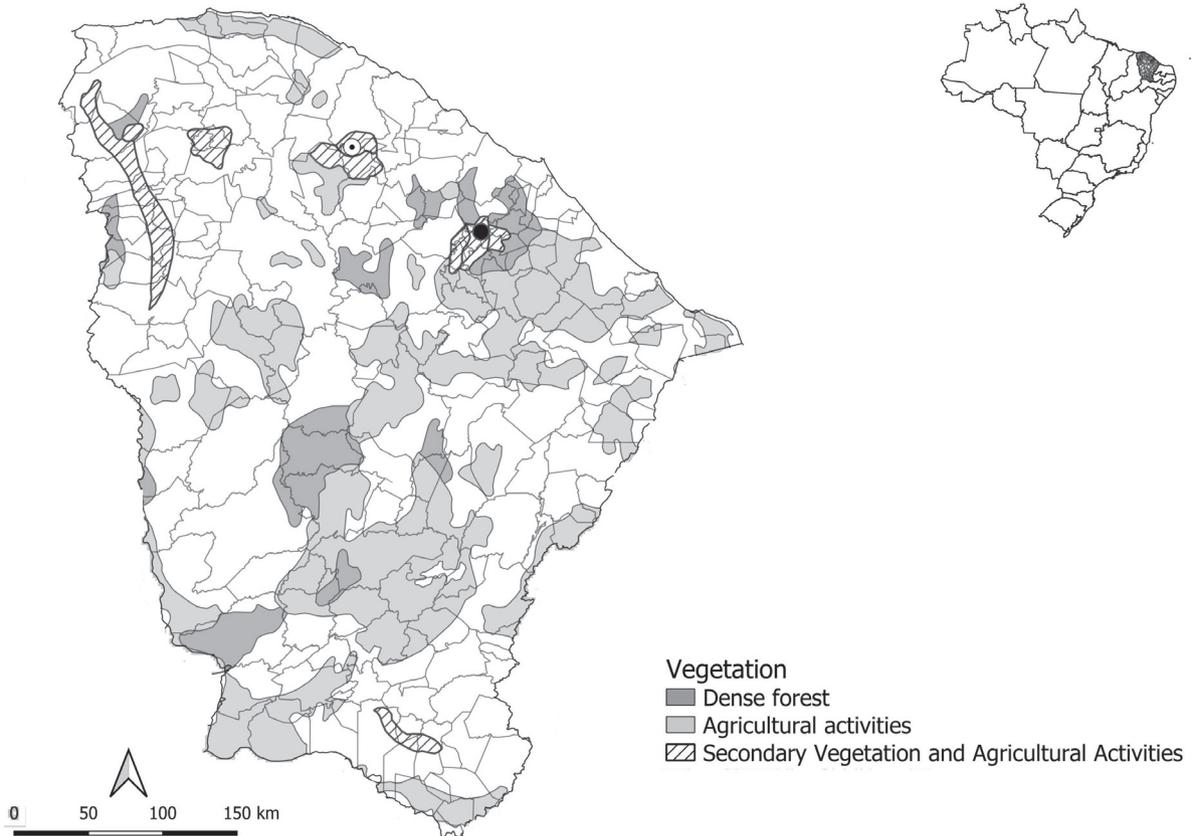


Figure 4 - Areas with autochthonous cases of Paracoccidioidomycosis in the Ceara State, Northeast of Brazil. The black dot shows the location of Palmacia city in Baturite mountain and the white halo with black dot locates the Uruburetama mountain, where the new case was described.

Paracoccidioides spp. infection was 32.1%¹¹. Double immunodiffusion and paracoccidioidin antigen skin testing were also performed in Palmacia, the area in which the first autochthonous case in Ceara was reported¹². A prevalence of 18.9% was observed and only one serology for *P. brasiliensis* was positive in the index case. Variations in the results of surveys involving intradermal testing can be due to the antigen and dilution differences used in the studies, aside from cross-reactions between histoplasmin and paracoccidioidin tests¹⁰. In an attempt to reduce these possible biases, the intradermal test using a 43-kDa glycoprotein (specific for *P. brasiliensis*) demonstrated 45.8% of positivity in Midwestern Brazil¹³.

Between 1998 and 2006, 6,732 hospitalizations occurred due to PCM in Brazil¹⁴. Admissions were recorded in 27% of the 5,560 Brazilian municipalities, although they took place in all 26 States of the country. In the Northeast region, a total of 687 admissions for PCM were registered. Maranhao (52.2%), Piaui (19.8%), Bahia (14%) and Ceara (8.3%) were the States with more hospitalizations due to PCM in this area¹⁴. Teresina, the capital of Piaui State, ranks tenth among the Brazilian cities with the highest number of admissions¹⁴. In spite

of its location in the semiarid Brazilian Northeast, there are growing areas of agribusiness in Southern Piaui. Furthermore, the city receives patients from several neighboring States.

In studies conducted in Maranhao (Brazilian Northeast)⁸, Espirito Santo¹⁵, Rio de Janeiro¹ and Sao Paulo¹⁶ (Southeastern Brazil), the chronic form was more frequently found in adult men and rural workers. The male/female ratio varies according to the geographic region, from 3:1 to 10:1 in the Brazilian Southeast and Midwest, respectively. Moreover, no racial predisposition was observed¹⁶. The most affected organs were oropharyngeal mucosa, upper airway tract, lungs, lymph nodes and skin. In the case reported herein, similar clinical forms affecting the nasal and palate regions were observed.

According to the Brazilian guidelines for the clinical management of PCM¹⁷, the chronic form is more common in men aged between 30 and 60 years old. The duration of symptoms is variable and can be greater than one year due to the lack of access to medical assistance or delay in the fungus diagnosis¹⁷. Smoking, high alcohol intake and malnutrition are factors that contribute to the progression of the disease^{18,19}.

Late diagnosis of this mycosis can lead to sequelae in different organs due to chronic inflammatory processes, fibrosis and deformities. Involvement of the upper respiratory tract may lead to complications such as dysphonia, vocal cord lesions and laryngeal obstructions¹⁹. In the present study, the patient presented a deformity in the hard palate, although no speech or swallowing impairment was reported.

The main differential diagnosis of PCM should be mucocutaneous leishmaniasis, endemic in Ceara. Nevertheless, painful oral lesions and hoarseness are mainly associated with PCM, whereas nasal obstruction is more related to mucocutaneous leishmaniasis²⁰. The present case report demonstrates both, oral and nasal involvement, uncommon in PCM. However, it was widely assessed by histopathology, culture, serology, and molecular testing, confirming the diagnosis of PCM.

The gold standard for PCM diagnosis is based on the fungal identification by direct visualization or culture of clinical samples or tissues. Double immunodiffusion can also be used in association with clinical features. However, the variation in antigen production, unavailability of commercial kits and the lack of validation of *P. lutzii* and cross-reactions with other fungal infections have limited the use of this test. Nonetheless, sensitivity ranged between 69-100% and specificity between 80-100%. Even though molecular tests, such as PCR, are expensive and only available at referral centers for mycoses sensitivity and specificity were high and enabled the species identification¹⁹.

Although PCM is uncommon and frequently neglected in Northeastern Brazil, it should be considered mainly in male adults and farmers from mountainous areas, with pulmonary or chronic mucosal lesions. It is noteworthy that human migration, expansion of agricultural borders, climate and environmental changes, and new agricultural and social practices have been causing an impact on the occurrence of *Paracoccidioides* spp. infection and PCM², a fact that may help explaining the identification of new areas with the presence of the fungus.

CONCLUSION

Paracoccidioides spp. infection should be considered in populations from the mountainous areas of Ceara. More studies are needed in the region to better elucidate the risk factors related to the presence of the fungus in the environment.

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AUTHORS' CONTRIBUTIONS

Matheus Alves de Lima Mota: data collection and compilation, literature review, article writing; Lisandra Serra Damasceno: data and text review, article writing; Silviane Praciano Bandeira: laboratory support, culture diagnosis and PCR, article writing; Terezinha do Menino Jesus Silva Leitão: data and text review, article writing.

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