

Case report

A case of imported paracoccidioidomycosis in a German legionnaire

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We report on a case of the chronic form of paracoccidioidomycosis with swelling and ulcerations of the mouth in a German legionnaire who also suffered from a chronic bronchitis. The patient had worked for many years in Brazil, an area endemic for the disease. Infection due to *Paracoccidioides brasiliensis* was diagnosed in Germany, more than 10 years after the patient's return. Diagnosis was established by the presence of yeast cells with multipolar budding in the tissue of the oral lesion. Furthermore, the fungus was grown in a liquid *Leishmania* culture medium. Identification of the fungus was based on morphology and genetic sequencing. Furthermore, IgG antibodies against a 43-kDa antigen of *P. brasiliensis* were detected in a western blot. After itraconazole therapy (400 mg day⁻¹) for 4 weeks, the lesions had disappeared almost completely, but the therapy was continued for further 5 months to avoid relapse of the infection.

Keywords German legionnaire, human infection, imported paracoccidioidomycosis, mucocutaneous ulceration, *Paracoccidioides brasiliensis*

Introduction

Paracoccidioides brasiliensis is the most important agent of systemic mycoses in Latin America. Diseases due to this fungus are geographically distributed in Central and South America with a high incidence in Brazil, Venezuela and Colombia. Recently, armadillos have been described as an animal host for this fungus [1]. Infections are probably acquired by inhalation of conidia from the soil. Diseases due to this fungus have been classified into a juvenile and an adult form [2]. The juvenile form is acute or subacute, whereas the adult form is usually chronic, with prolonged latency periods of many years or decades between infection and clinical manifestation.

Respiratory symptoms may be non-specific. The chronic multifocal form is frequently characterized by pulmonary involvement and mucocutaneous lesions, predominantly in the oral and nasal cavity.

Because of a high tropism towards the adrenal glands, systemic paracoccidioidomycosis is the most frequent aetiology of Morbus Addison in endemic areas [3]. Symptoms caused by cerebral lesions may be the first clinical manifestation of paracoccidioidomycosis. The disease has been also called South American blastomycosis and Lutz-Splendore-Almeida's disease. Here, we present a case of imported paracoccidioidomycosis that was diagnosed in Germany.

Case history

In January 2000, a 61-year-old German man arrived to a hospital in the south of Germany with a 10-year history of swellings and ulcerations of his mouth. From

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1970 to 1980, the patient worked as a legionnaire in Asia and Africa; from 1980 to 1990, he lived in South America, mainly in the region of Mato Grosso (Brazil). Since 1990 he had been living in Germany. Several years previously, a tropical pulmonary mycosis was diagnosed, according to the patient's statement, and he had been suffering from a chronic bronchitis for many years. He admitted that he had been addicted to alcohol in the past, mainly during his military activities. In 1995, a Billroth II operation was performed because of relapsing gastric ulcerations. Five years previously the patient had been treated in hospital because of a therapy-refractive pyogenic ulceration surrounded by an oedematous erythema at the left angle of his mouth. At that time, *Staphylococcus aureus* was isolated from wound swabs. Histological examinations showed granulomatous lesions as well as abscess formation. After antibiotic treatment the lesion healed and the patient was discharged. In 1997, a biopsy of the bone marrow was performed to exclude a gammopathy. The clinical picture in January 2000 showed erythematous and swollen lips, mainly on the left side, as well as mucocutaneous pustules and ulcerations with pus. At the hard palate and under the patient's tongue more livid nodules surrounded by whitish plaques could be observed (Fig. 1). The lesions were very painful and the patient was physically and psychologically handicapped, so that he rarely left his home and avoided social contact. Clinical symptoms included occasional night sweats. Clinico-chemical parameters showed microcytic anaemia and elevation of the erythrocyte sedimentation rate (ESR: 24 mm); other tests gave normal results. Because of the clinical picture and the long residence in tropical areas a leishmaniasis was assumed, but serology for *Leishmania* was negative. A biopsy was therefore taken from the lesions and sent for culturing of

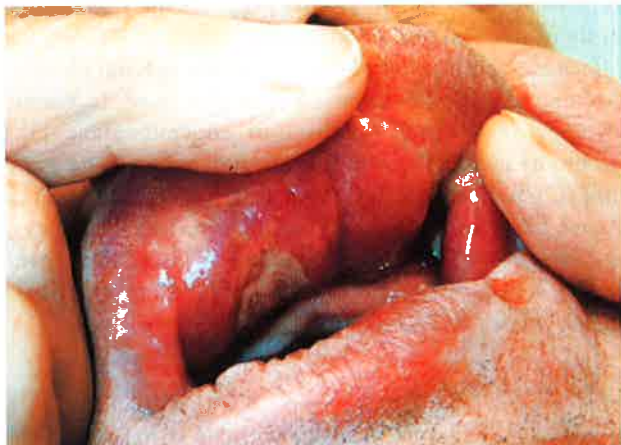


Fig. 1 Swollen lips, mainly on the left side, and ulcerations as well as mucocutaneous lesions, surrounded by whitish plaques.

Leishmania and for histological examination. With periodic acid-Schiff (PAS) staining yeast-like fungal elements were recognized in the tissue. Two weeks later, a fungus rather than *Leishmania* promastigotes was growing in the liquid *Leishmania* culture medium (Leibovitz L15, Bio Whittaker, B, Belgium). Assuming that the infection was due to a thermally dimorphic pathogen, physicians prescribed itraconazole (400 mg day^{-1}). After 4 weeks, the lesions had disappeared almost completely, but therapy was continued for another 5 months. At the beginning of 2000, immunodiffusion (ID) tests were applied to detect antibodies against dimorphic fungi using antigens of *Blastomyces dermatitidis*, as well as *Coccidioides immitis* and *Histoplasma capsulatum* (Immuno-Mycologics, Inc., Norman, OK, USA). All these tests gave negative results.

Mycology

Biopsy of the oral ulcerations revealed a granulomatous reaction and the presence of budding yeast cells with adjacent multinuclear giant cells. The yeast ranged from 3 to 6 μm in diameter, with only sparse multipolar budding, which is a diagnostic criterion for paracoccidioidomycosis (Fig. 2). The liquid *Leishmania* culture medium was incubated at 26°C . After 1 week, whitish mycelial growth could be observed. Subcultures were made on brain-heart infusion agar (BHI) at 30°C and 37°C . After 1 week on BHI incubated at 37°C , cream-coloured, yeast-like wrinkled colonies could be observed. Rounded yeast-like cells with multiple budding were seen microscopically. The cells were thick walled and birefringent. The daughter cells differed in size and were connected to each other by small bridges, typical of

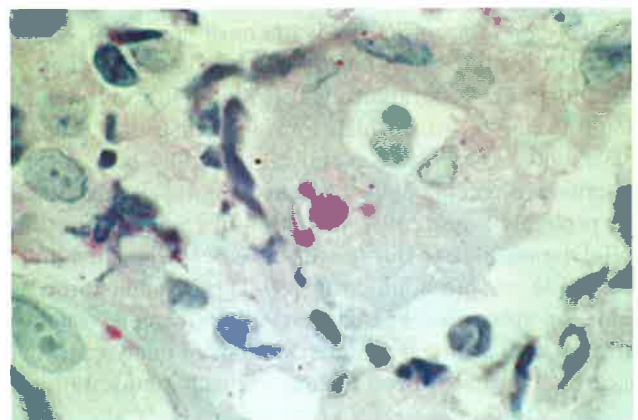


Fig. 2 Histopathology of the biopsy with granulomatous reaction, multinucleated giant cells and one *Paracoccidioides brasiliensis* yeast cell with sparse multipolar budding (periodic acid-Schiff staining, $\times 100$ objective).

P. brasiliensis. At 30 °C the culture needed one further week until a sterile whitish mould with short aerial hyphae could be recognized. Microscopic examination showed septate, branched hyphae with terminal or intercalary chlamydospores. The fungal isolate was used directly for molecular identification by DNA sequencing. Sequence analysis was performed of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, including the internal transcribed spacer 1 (ITS1), the 5.8S ribosomal DNA and ITS2. For this purpose, DNA was extracted by sonification in cetyltrimethylammoniumbromide (CTAB) buffer [4] containing glass beads and amplified with the primer pair ITS5/ITS4 [5]. The polymerase chain reaction (PCR) product was purified using the PCR Extraction Kit (Qiagen, Hilden, Germany) and sequenced directly using the primers described. DNA sequencing was performed by the dideoxynucleotide termination method, using the dye deoxy terminator chemistry and the ABI 377 automated sequencer (AB Applied Biosystems, Weiterstadt, Germany). Results were compared with GenBank sequences using the BlastN 2.1.1 programme. One hundred per cent similarity was obtained with ITS sequences of *P. brasiliensis*. The strain was taken into the Centraalbureau voor Schimmel (CBS; Utrecht, The Netherlands) culture collection as CBS 109819.

Discussion

Imported paracoccidioidomycoses are extremely rare in Germany, as well as in several other European countries [6–10]. The Robert Koch-Institut, Berlin, has been involved in the diagnosis of two other proven cases of paracoccidioidomycosis diagnosed in Germany between 1994 and 2000. Both patients were male, 22 and 58 years old, who had been infected in Brazil and Ecuador (unpublished). The clinical history of our patient is typical of the chronic form of paracoccidioidomycosis. Oral ulceration is one of the main complaints of patients with paracoccidioidomycosis when they are seeking medical help [11]. One can speculate that the patient's 'tropical pulmonary disease' some years previously was the first clinical symptom of pulmonary infection due to *P. brasiliensis*. The pyogenic ulceration of the mouth 5 years previously had probably also been a symptom of this infection, superinfected by *S. aureus*. In addition, it could not be excluded that the relapsing gastric ulcerations seen in 1995 and the assumed gammopathy were also symptoms of the spreading or relapsing of paracoccidioidomycosis. In an overview of 13 imported paracoccidioidomycoses diagnosed in Japan, some cases were found to involve both the stomach and bones [12].

We were not able to obtain any more detailed information about the tropical pulmonary mycosis from either the patient or his family doctor. Unfortunately, detection of antibodies against *P. brasiliensis* was not performed until March 2001 because the amount of serum sent for microbiological examination was not sufficient for a full range of tests, and the patient failed to cooperate and was lost before further specimens could be taken. At that time, the anti-*P. brasiliensis* ID test was negative, although IgG antibodies against the diagnostic 43 kDa antigen of *P. brasiliensis* could be detected using Western blotting. This fraction has been found to be highly specific for paracoccidioidomycosis [13]. The negative result in the ID test might be due to the long period of treatment and the higher sensitivity of Western blot compared with the ID test. As shown in this case, molecular tools can speed the identification of isolates highly suggestive of a thermally dimorphic fungus.

Itraconazole is highly active *in vitro* against *P. brasiliensis* and is the drug of choice for oral therapy. However, treatment failures have been reported [14]. Amphotericin B may be the most effective alternative. In any case, successful treatment needs long-term administration of antifungal drugs. Although the lesions in our patient resolved almost completely after 4 weeks of oral treatment with itraconazole, therapy was continued for a further 5 months because of the common occurrence of relapses. Because of the restricted geographic distribution of *P. brasiliensis*, it can be deduced that the primary infection with this fungus probably happened 10–20 years ago while the patient was stationed in Mato Grosso. His occupation could have led to close contact with soil particles contaminated with the infectious agent.

This case shows that clinicians should be aware of the extended latency between the infection and the mucocutaneous manifestation in the chronic form of paracoccidioidomycosis. In non-Latin American countries, one should also be aware of the importance of the patient's travel history.

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