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Chromoblastomycosis caused by *Rhinocladiella aquaspersa*

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A B S T R A C T

A case of chromoblastomycosis of the hand caused by *Rhinocladiella aquaspersa* is described. The case was acquired locally in tropical Venezuela and was successfully treated with oral itraconazole.

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1. Introduction

Chromoblastomycosis is a chronic granulomatous fungal infection that causes hyperproliferation of cutaneous and subcutaneous tissues and is histologically characterized by the presence of muriform cells. Infection originates after the etiologic agent gains entrance via percutaneous trauma [1]. Main agents responsible for its etiology are *Fonsecaea pedrosoi*, *Fonsecaea monophora*, *Cladophialophora carrioni*, *Phialophora verrucosa*, and *Rhinocladiella aquaspersa*. The disease is found in tropical and subtropical areas [2–4], with the vicarious agents [5] of *Fonsecaea* and *Phialophora* occurring under hot and humid conditions [6], and *Cladophialophora* being restricted to arid climates [7–10]. *R. aquaspersa* is an uncommon cause of chromoblastomycosis, with most cases having been reported from the American continent [11–14]. The present paper presents a further case from Venezuela and summarizes the climatic conditions of the areas where *R. aquaspersa* is endemic.

2. Case report

A 63-year-old male construction worker, native and resident of a rural area in Siquisique (Caserío La Unión), Lara State, Venezuela, presented with an asymptomatic, localized lesion affecting the dorsal surface of the left hand, involving the knuckles and proximal interphalangeal joints of the third and fourth fingers. The dermatosis produced retraction of the fifth finger. Examination of the skin disclosed a scaly, crusty, dull-red plaque, friable with hemorrhagic dots. The lesion was almost flat and eroded. According to the patient, the lesion had developed over an 18-month period and trauma or inoculation was not recalled. There were no lymphatic commitments. He has never been outside Siquisique city. The patient denied other problems but was in poor health and nutritional conditions. To recover the etiologic agent, the area was soaked with alcohol and recovered the etiologic agent, the area was soaked with alcohol and was successfully treated with oral itraconazole.

Microscopic examination of a biopsy specimen from the lesion stained with periodic acid Schiff showed pseudoepitheliomatous hyperplasia and a mixed cell granulomatous infiltrate consisted of lymphocytes, histiocytes, giant cells, and neutrophils. Muriform cells were observed in the granulomatous tissue. These results
confirmed the clinical diagnosis of chromoblastomycosis (Fig. 1).

Cultures of skin scrapings were performed on Sabouraud’s glucose agar (SGA) with chloramphenicol (0.5 mg/ml). After incubation at 30°C a slow-growing dark mold was recovered. Microscopically scarce conidia were observed which did not allow identification of the fungus. The isolate was submitted to the Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico, for morphological and molecular identification. The patient was treated with oral itraconazole (100 mg/day). The lesions improved after 2 months, with significant healing, desquamations having disappeared, and culture became negative. No side effects were noted by the patient during the course of treatment. Unfortunately, the patient could not be contacted for further follow-up.

2.1. Mycology

The mold was subcultured on SGA and on potato dextrose agar (PDA) on culture plates (20 mm diam.) incubated at 30°C. After 2 weeks velvety colonies developed which were dark olive with an olivaceous black reverse. Slide cultures were performed on PDA blocks. After 2 weeks slides were stained with lactophenol cotton blue and evaluated in a Nikon Eclipse 50i microscope. Inocula suspension of 10^6 CFU/ml were prepared by hemacytometric count and 0.2 ml of each dNTP, 200 nM of each primer, 1 U of AmpliFaq polymerase (Bioline, Randolph, MA, U.S.A.) and 100 ng DNA. The thermocycling conditions were: 94°C for 4 min, followed by 30 cycles of 94°C for 60 s, 55°C for 90 s, and 72°C for 90 s, with final extension at 72°C for 5 min. The final products were electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

Amphotericin B (AMB) (Bristol-Myers Squibb, Princeton, NJ, U.S.A.); itraconazole (ITZ) (Janssen-Pharmaceutica, Beerse, Belgium); voriconazole (VZ) (Pfizer, Inc., New York, NY, U.S.A.); posaconazole (PSZ) (Scherer-Plough, Kenilworth,N.J., U.S.A.); and terbinafine (TB) (Novartis, Mexico) were obtained in reagent-grade powder form from their respective manufacturers. Isolates were evaluated by using Clinical and Laboratory Standard Institute broth macrodilution approved standard reference method M38-A2 [21]. After adequate sporulation occurred on PDA, the mycelium was overlaid with sterile distilled water, and suspensions were made by softly scraping the colonies with wooden applicators. Heavy fragments were allowed to settle, and the supernatant was transferred to sterile tubes. Inocula suspension of 10^6 CFU/ml were prepared by hemacytometer counts and then diluted in RPMI 1640 medium with glutamine and morpholinopropanesulphonic acid buffer at a concentration of 0.165 M (Angus, Niagara Falls, NY, U.S.A.) to obtain a final organism...
concentration of $0.4 - 5 \times 10^4$ CFU/ml. The inoculum size for all tests was verified by plating 10 μl onto PDA, incubating the plates at 35 °C, and counting the number of colonies. Serial dilution of the drugs was made following the CLSI guidelines in order to obtain final concentrations of the drugs ranging from 0.015 to 8 mg/L for all antifungal compounds. *Candida krusei* ATCC 6258 and *Paecilomyces variotii* ATCC MYA3630 were used for quality control.

Results of susceptibility testing were: AMB = 2 μg/ml, TBF = 0.06 μg/ml, ITZ = 0.125 μg/ml, VRZ = 1 μg/ml, and PSZ = 0.25 μg/ml, respectively.

### 3. Discussion

*R. aquaspersa* is a rare agent of human chromoblastomycosis. An overview of clinical cases is presented in Table 1; several reports provided only very scant information. The majority of historical cases have not been verified by sequence data, but the morphology of *R. aquaspersa* is unmistakable. Most cases report the presence of murriniform cells in tissue, but in Case 3 these were absent. The clinical appearance of the disease is highly diverse [23]. The main agents of the disease are classified in *Cladophialophora* and *Fonsecaea* and are restricted to arid and tropical climates, respectively [5,9], but environmental data of cases of *R. aquaspersa* do not show any consistency (Table 1). Caserio La Unión of the present case is located in the northernmost part of the state, between 600 and 700 m above sea level. This area presents temperatures between 23 and 28 °C, its annual precipitation varies between 950 and 1400 mm, and its annual evaporation rates range from 1400 to 2400 mm. The predominance of semi-deciduous vegetation is associated to the aforementioned climatic conditions. The global distribution of *R. aquaspersa* is (sub)tropical. The species has thus far not been recovered from the environment, but Badali et al. [14] mentioned a case acquired by from a cactus thorn suggesting a natural habitat on living or dead plants. Marques et al. [13] reported a case secondary to an insect bite.

The species is susceptible to commonly used azoles, and in our case treatment of the infection with itraconazole led to healing within two months.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgments

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### References


### Table 1

Published cases of *Rhinocladiella aquaspersa*.

<table>
<thead>
<tr>
<th>Case</th>
<th>Strain</th>
<th>Patient, age</th>
<th>Site, duration</th>
<th>Geography</th>
<th>Therapy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CBS 313.73</td>
<td>Male 56</td>
<td>Hand, 15 yr</td>
<td>Mexico</td>
<td>Alupye, Morelos, Mexico, 1000 m alt, arid</td>
<td>Terbinafin 6 mo (500 mg/d), cured</td>
<td>[24]</td>
</tr>
<tr>
<td>2 CBS 122635</td>
<td>Foot, cactus</td>
<td>Foot, cactus</td>
<td>Solanea, Paraiba, Brazil, 600 m alt, tropical</td>
<td>MRZ = 0.25 μg/ml, VRZ = 1 μg/ml, PSZ = 0.25 μg/ml, respectively.</td>
<td>Electroacuteration, itraconazole (200 mg/d), cured</td>
<td>[14]</td>
</tr>
<tr>
<td>3 FMR 7699</td>
<td>Female 62</td>
<td>Ear, 5 yr</td>
<td>Medellín, Colombia, 1400 m alt, temperate dry</td>
<td>Itraconazole 7 mo (200 mg/d), cured</td>
<td>[11]</td>
<td></td>
</tr>
<tr>
<td>4 Male 60</td>
<td>Abdomen</td>
<td>South Korea</td>
<td>Itraconazole 4 mo (200 mg/d), surgery, cured</td>
<td>Ketoconazole, failure</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td>5 Female 52</td>
<td>Arm, leg, forehead, 2 y, insect bite</td>
<td>Maranhão, Brazil, 300 m alt, tropical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Male 52</td>
<td>Upper limb, 2 yr</td>
<td>Santa Cruz de Bucaral, Falcon state, Venezuela, 800 m, arid</td>
<td>Itraconazole; failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Male 5</td>
<td>Costa Rica, tropical</td>
<td>MINAS GERIAIS, Brazil, 300 m alt, tropical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 No data</td>
<td>Costa Rica, tropical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 No data</td>
<td>Siquisique, Lara State, Venezuela, 600 m alt, tropical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 CBS 132913</td>
<td>Male 63</td>
<td>Hand, 18 mo</td>
<td></td>
<td>Itraconazole 2 mo (100 mg/day), cured</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


