Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Tropica 98 (2006) 277–285
ACTA TROPICA
www.elsevier.com/locate/actatropica

Leishmania (*Viannia*) *braziliensis* is the prevalente species infecting patients with tegumentary leishmaniasis from Mato Grosso State, Brazil

Maria de Lourdes Ribeiro Carvalho [a], Antero Silva Ribeiro de Andrade [b], Cor Jesus Fernandes Fontes [c], Márcia Hueb [c], Soraia de Oliveira Silva [a], Maria Norma Melo [a,*]

[a] *Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brazil*
[b] *Comissão Nacional de Energia Nuclear, Centro de Desenvolvimento de Energia Nuclear, Avenida Antônio Carlos 6627, 31120-970 Belo Horizonte, MG, Brazil*
[c] *Hospital Universitário Júlio Muller, Universidade Federal do Mato Grosso, Rua Luis Philippe Pereira Leite S/N, 78048-790 Cuiabá, MT, Brazil*

Received 2 December 2005; received in revised form 7 May 2006; accepted 1 June 2006

Abstract

The frequency of *Leishmania* (*Viannia*) *braziliensis* infection among patients of Mato Grosso, Brazil was estimated by polymerase chain reaction—PCR, DNA hybridization and by isoenzyme electrophoresis. Analysis of DNA polymorphism was carried out using random amplified polymorphic DNA-PCR (RAPDPCR) with five different primers. The patients were attended from May 1997 to February 1998 at the Reference Ambulatory for American Tegumentary Leishmaniasis at Júlio Müller University Hospital of the Federal University of Mato Grosso, Brazil. In a first screening by PCR and DNA hybridization 94.1% of 68 patients, from whom parasites were isolated in culture medium, were found to be infected with species of the *Le. braziliensis* complex. Only four patients (5.9%) were infected with species of *Le. mexicana* complex. Thirty-three samples of *Le. braziliensis* complex and three of *Le. mexicana* complex were typed by isoenzyme analysis as *Le.* (*V.*) *braziliensis* sensu stricto and *Le. (Leishmania) amazonensis*, respectively. The predominant species was *Le.* (*V.*) *braziliensis*, although most of the patients of this study came from the northern area of Mato Grosso, which is part of the Amazonian region of Brazil, where other known species of both subgenus Viannia (*Le. braziliensis* complex) and Leishmania (*Le. mexicana* complex) are present. The results of RAPD showed higher genetic variability among the *Le.* (*V.*) *braziliensis* samples from Mato Grosso. The importance of these results concerning the taxonomic status of New World Leishmania, and their implications for both clinical and epidemiological data is discussed.

Keywords: American tegumentary leishmaniasis; Mato Grosso; Brazil; *Leishmania* (*Viannia*) *braziliensis*

1. Introduction

American tegumentary leishmaniasis (ATL) in Brazil is a parasitic disease caused by at least seven species of the genus *Leishmania*, members of the *Le. braziliensis* and *Le. mexicana* complexes. *Le.* (*V.*) *braziliensis*

* Corresponding author. Tel.: +55 31 3499 2969; fax: +55 31 3499 2970.
E-mail address: melo@icb.ufmg.br (M.N. Melo).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.06.001

is considered the most frequent agent causing ATL in the Americas and it is the most important *Leishmania* species in Brazilian foci out of the Amazonian region (Lainson et al., 1994). Lately, ATL cases have increased in practically all States of Brazil, and have also expanded their geographical distribution. From 1985 to 2003, 523.975 cases of tegumentary leishmaniasis were recorded in Brazil (Basano and Camargo, 2004). In the State of Mato Grosso, where the patients included in this study reside, the annual number of ATL cases has been increasing progressively and its incidence has exceeded 200 cases per 100,000 inhabitants (FUNASA, 2004).

The main clinical characteristics and some epidemiological aspects of tegumentary leishmaniasis in Mato Grosso were described by Hueb (1997) and Carvalho et al. (2002). The Ministry of Health has characterized the distribution and frequency of leishmaniasis in the State but it has no information about the dynamics of transmission, clinical behavior and, mainly, the species involved. The accurate identification of *Leishmania* species has clinical and epidemiological benefits. It allows better orientation of the medical treatment and follow-up, since clinical manifestation and also parasite sensibility to drugs may vary depending on the species of *Leishmania*. Detection of the particular *Leishmania* species from an endemic area is also essential to plan more suitable control activities and to understand the epidemiology of the disease.

The aim of the present study was to identify what species of *Leishmania* are prevalent in the patients with ATL who live in endemic areas of the State of Mato Grosso and were attended at the Reference Ambulatory for ATL of Júlio Müller University Hospital/Federal University of Mato Grosso. Clinical and laboratory criteria were used to diagnose ATL (Carvalho et al., 2002). As a first screening, we used PCR and DNA Hybridization protocols able to differentiate between the *Le. braziliensis* and *Le. mexicana* complexes. Enzyme electrophoresis was used to identify *Leishmania* at the species level, and RAPD-PCR to analyze and compare the DNA polymorphism of *Le.* (*V.*) *braziliensis* between samples from the State of Mato Grosso and samples of the same species isolated from patients from the State of Minas Gerais, which greatly differs ecologically from the State of Mato Grosso.

2. Material and methods

2.1. Sample collection

Biopsies were taken from lesions of patients presenting exclusively cutaneous lesions, the majority ulcerated with 3–4 months of evolution. Besides the presence of the typical lesion, they also presented positive results to the Montenegro skin test and indirect fluorescent antibody test-IFAT (data not shown). The patients were from rural areas and were attended at Júlio Müller Hospital, a reference medical service for leishmaniasis, located in Cuiabá (Mato Grosso). Most of the patients were infected in the Amazon Forest in the north of Mato Grosso State (Fig. 1).

The research protocol using human subjects in this study has been reviewed and approved by the Ethics in Research Committee of the University Hospital Júlio Müller-Federal University of Mato Grosso. Informed consent was obtained from all patients. The cutaneous lesions were prepared in the normal manner for biopsy, which includes antisepsis and anesthesia. The biopsies were taken with a sterile punch from the raised border of the lesion and inoculated in Evans biphasic medium (Evans, 1993). The positive cultures were cryopreserved at −196 °C under liquid nitrogen.

2.2. Polymerase chain reaction—PCR

The PCR reactions were performed using the primers B1/B2, specific for the *Le. braziliensis* complex (Bruijn and Barker, 1992) and M1/M2 specific for the *Le. mexicana* complex species (Eresh et al., 1994), that amplify products of 750 and 730 bp, respectively. An osmotic promastigote lysate (10^4 cells/20 μl) of each sample was obtained and 2.0 μl were used in the reaction. Positive and negative controls were made using the WHO reference strains IFLA/BR/1968/PH8 – *Le.* (*L.*) *amazonensis* and MHOM/BR/1975/M2903 – *Le.* (*V.*) *braziliensis*. A reaction tube with a final volume of 10 μl contained: 2.0 μl of the culture lysate, 25 mM KCl, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.0), 10 pmol of each primer and 2.5 units of Taq DNA Polymerase (CENBIOT-RG-Brazil). Samples were overlain with light mineral oil and initially denatured at 96 °C, for 6 min. Cycles consisted of annealing at 67.5 °C for 1 min, extension at 72 °C for 1 min and denaturation at 93 °C for 0.5 min. Programmes were run for 25 cycles. Amplification products (10 μl) were analysed by electrophoresis in 1% agarose gels in TBE buffer (89 mM Tris Borate, 12 mM EDTA pH 8.0). The molecular marker weight used was ϕ × 174-Hae III (Promega).

2.3. DNA hybridization

The cryopreserved cultures were grown in minimum essential medium-MEM (GIBCO-BRL Life Technologies Inc., Gaithersburg, MD), supplemented with 15%