Transactions of the Royal Society of Tropical Medicine and Hygiene 106 (2012) 419–423

Contents lists available at SciVerse ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Occurrence of *Trypanosoma caninum* in areas overlapping with leishmaniasis in Brazil: what is the real impact of canine leishmaniasis control?

J.H.S. Barros[a,*], A.B.P.F. Almeida[b], F.B. Figueiredo[c], V.R.F. Sousa[b], A. Fagundes[a], A.G.S. Pinto[a], C. Baptista[a], M.F. Madeira[a]

[a] *Laboratório de Vigilância em Leishmanioses, Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ, Brasil*
[b] *Departamento de Clínica Médica Veterinária, Faculdade de Agronomia, Medicina Veterinária e Zootecnia, Universidade Federal do Mato Grosso, Av. Fernando Correa da Costa, S/N, campus da UFMT, Boa Esperança, Cuiabá, MT, Brasil*
[c] *Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos, Instituto de Pesquisa clínica Evandro Chagas, Fundação Oswaldo Cruz, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, RJ, Brasil*

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 30 March 2012
Accepted 30 March 2012
Available online 10 May 2012

Keywords:
Trypanosomatidae
Dogs
18S rRNA gene
Nested-PCR

ABSTRACT

Trypanosoma caninum is a parasite of the *Trypanosoma* genus recently described in the natural infection of dogs in the municipality of Rio de Janeiro, Brazil. Suspecting the existence of a natural cycle and the circulation of this new species, the objective of this study was the taxonomic identification of samples of *Trypanosoma* spp. isolated from dogs in different Brazilian regions. Parasites were solely obtained from skin fragments culture and characterized by nested-PCR targeting the partial sequence of 18S rRNA gene and PCR products were sequenced. Thirty-three samples, obtained in São Paulo, Minas Gerais, Goiás, Mato Grosso and Rio de Janeiro states were analyzed. PCR and sequencing showed that the isolates were genetically identical or closely similar and confirmed *T. caninum* identity. This report broadens the geographical distribution of *T. caninum* in Brazil and discusses the impact of the presence of this parasite in areas of canine leishmaniasis occurrence.

1. Introduction

The genus *Trypanosoma* includes a complex group of parasites that infect a broad range of hosts worldwide. According to the species, they may infect blood or other tissues, under the trypomastigote or amastigote forms, respectively.[1] Recently, a new species, *Trypanosoma caninum*, was described in domestic dogs in the state of Rio de Janeiro, Brazil, isolated into culture from intact skin fragments; attempts to isolate it from blood or other tissues from naturally infected dogs did not succeed.[2,3] Aspects related to the biological forms present in the vertebrate host and its natural cycle are still unknown. However, molecular characterisation shows it is not related to *T. cruzi* or *T. rangeli*, and analysis of partial SSU ribosomal DNA sequences give *T. pestanai* and a wombat trypanosome as the closest matches.

In Brazil, visceral leishmaniasis (VL) is a serious public health problem and the domestic dog is one of the targets for control actions because it is considered a major reservoir of *Leishmania* (*L.*) *chagasi* (= *L. infantum*), the VL etiological agent.[4] The Brazilian leishmaniasis control program recommends diagnosis and euthanasia of sera reactive dogs as control measures for VL.[5] In this context, the presence of other trypanosomatids, besides *Leishmania* parasites, infecting domestic dogs in overlapping areas,

* Corresponding author. Tel.: +55 21 21 38659541.
E-mail address: juliana.helena@ipec.fiocruz.br (J.H.S. Barros).

0035-9203/$ – see front matter © 2012 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.trstmh.2012.03.014

may constitute a confounding factor in canine diagnosis, since surveys are based on serological tools for the identification of positive animals.

Our team has monitored *Leishmania* species and the genetic variants that may be infecting domestic dogs,[6] and samples of *Trypanosoma* spp. were isolated during these studies from different Brazilian endemic areas. Morphological and biological aspects of these isolates show patterns very similar to *T. caninum*, however, the correct identification of these isolates is fundamental, since they come from VL endemic areas and evaluating the impact that *T. caninum* has in those areas is still a great challenge. Thus, the objective of this study was to characterize 33 samples of *Trypanosoma* spp. isolated from dogs and report the presence of *T. caninum* in different Brazilian regions, where cases of canine VL occur.

2. Materials and methods

2.1. Samples

The samples for this study were obtained during surveys of domestic dogs, the objective of which was the diagnosis of canine VL conducted in the states of Rio de Janeiro, comprising the municipalities of Rio de Janeiro, Niterói and Maricá; São Paulo (Bauru); Minas Gerais (Belo Horizonte); Mato Grosso (Cuiabá) and Goiás (Brasília). In each of these regions dogs were randomly evaluated, dogs of both sexes and aged six months or more being sampled. Only housed dogs participated in the study, and after clinical examination intact skin fragments were collected for parasite isolation in culture and molecular analysis by PCR.

2.2. Isolation of parasites from dogs

Three approximately 3 mm sized skin fragments were collected from each animal; two were culture processed and one was frozen at −20 °C for molecular studies. For isolation in culture the skin fragment was immersed in saline containing 100 μg of 5′fluorocytocine, 1000 IU of penicillin and 200 μg of streptomycin per milliliter and stored at 4 °C for 24 h according to the protocol described by Madeira et al.[7] After this period, each fragment was transferred aseptically to a biphasic culture medium NNN (Novy-Neal-Nicolle) and Schneider's *Drosophila* medium (Sigma, St. Louis, MO, USA) containing 10% of fetal calf serum, incubated at 26–28 °C and examined weekly for a maximum of 50 days. The parasites isolated were maintained in the same culture medium, weekly subcultured and expanded in Schneider's medium until reaching about 10^9 parasites/mL. The parasites were harvested and twice washed in PBS pH 7.2 and the pellets stored in liquid nitrogen to be used in molecular assays.

2.3. Molecular analysis

Genomic DNA of cultured trypanosomes was extracted using DNAzol (Invitrogen, Carlsbad, CA, USA) and for skin samples, the DNA was extracted using Wizard® Genomic DNA purification kit (Promega, Madison, Wisconsin, USA). The manufacturer's instructions were followed in both procedures. The PCR amplifications were perfomed in a nested-PCR that targeted a partial sequence of the 18S rRNA gene using oligonucleotides and reaction conditions as described by Smith et al.[8] External primers TRY927F (5′ GAAACAAGAAACACGGGAG 3′) and TRY927R (5′ CTACTGGGCAGCTTGGA 3′) were used in the first round and internal primers SSU561F (5′ TGGGATAACAAAGGAGCA 3′) and SSU561R (5′ CTGAGACTGTAACCTCAAAGC 3′) were used in the second round. The PCR products were run on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. After PCR, the amplified products obtained in the second round with cultured forms or skin fragments were purified using the QIAquick Purification Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions and the nucleotide sequences were determined with an automatic sequencer (3730 DNA Analyzer, Applied Biosystems, Carlsbad, CA, USA). All nucleotide sequences obtained in this study were edited by the Bioedit program (BioEdit Sequence Alignment Editor version 7.0.5.2, Ibis Therapeutics; Carlsbad, CA, USA)) and analyzed by the Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The similarity percents were obtained comparing the 33 isolates with the sample of *T. caninum* (stock A27) already deposited in the GenBank (Accession no. GU385824).[3] Using the ClustalX program,[9] all sequences studied were aligned and compared each other and with sequences of *T. caninum* and other trypanosomatids available in GenBank.

3. Results

3.1. Samples

Thirty-three samples of *Trypanosoma* spp. were isolated from dogs in the states of São Paulo (Bauru, n = 6), Minas Gerais (Belo Horizonte, n = 3), Goiás (Brasília, n = 3), Mato Grosso (Cuiabá, n = 12) and Rio de Janeiro (Rio de Janeiro, n = 7, Niterói, n = 1 and Maricá, n = 1) during the surveys conducted between 2005 and 2011. All samples were obtained from intact skin cultures, and the geographical location of the animals is shown in Figure 1.

All isolates showed similar morphological aspects in culture, with exuberant growth in NNN/Schneider's medium, although seven samples were lost due to secondary contamination or because of reduced growth through the subcultures of the initial isolate. For this reason, samples of the skin of the animals whose cultures were lost were processed by PCR, using the same protocol as the cultured forms.

3.2. PCR and data analysis

The results of PCR assays showed the same amplification pattern for all 33 samples (26 from culture and 7 from skin), with product size of approximately 900 bp for the first round and 700 bp for the second round. The sequencing of the partial 18S rDNA showed that the isolates were genetically identical or closely similar and confirmed *T. caninum* identify. The similarity percents of the isolates and the access number in GenBank are shown in Table 1.