

Report of new cases of *Trypanosoma caninum* in Brazil

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ABSTRACT

Introduction: *Trypanosoma caninum* is a protozoan species recently described in dogs, whose occurrence has been reported in areas of overlap with visceral leishmaniasis. **Methods:** *Trypanosoma* sp. were isolated from nine dogs and characterized by molecular methods. **Results:** PCR and sequencing confirmed the presence of *T. caninum* in all dogs, revealing two new areas of transmission: Barra Mansa and São João do Piauí. **Conclusions:** The nine new cases described, when added to those already published, account for 62 cases of natural infection by *T. caninum* and show the geographical spread of this species to new areas, where other trypanosomatids circulate.

Keywords: *Trypanosoma caninum*. São João do Piauí. Barra Mansa.

Domestic dogs are naturally infected with different species of trypanosomatids^{(1) (2) (3) (4)}. These animals are of particular interest with regard to the transmission cycle of *Leishmania chagasi* and *Trypanosoma cruzi*, because they are considered reservoirs for both agents and enhancers of transmission in the peridomicile, and consequently a risk factor for the human population^{(3) (4)}. *Trypanosoma caninum* is the most recently described species of the *Trypanosoma* genus affecting dogs⁽⁵⁾, with 53 cases confirmed in different regions of Brazil⁽⁶⁾ prior to this present study. The species has unique biological characteristics, including its isolation exclusively from intact skin fragments^{(5) (7)} and the presence of aflagellate epimastigote forms in axenic cultures⁽⁸⁾. It appears to be nonpathogenic to dogs, in which the infection triggers a mild humoral immune response^{(7) (9)}. However, the presence of *T. caninum* in areas overlapping with those affected by canine visceral leishmaniasis (CVL) has been identified as a possible confounding factor for the accurate diagnosis of CVL. Thus, such overlap may have a negative impact on the control of this disease⁽⁶⁾. Culture-based testing has been an important tool for the diagnosis of *T. caninum* infection, although recent studies have demonstrated that polymerase chain reaction (PCR)-based tests that amplify specific regions of the 18S ribosomal DNA (rDNA) gene may

be a valuable additional tool for diagnosis, particularly in areas overlapping with those affected by *Leishmania chagasi*⁽¹⁰⁾.

Herein we report nine new cases of natural infection by *T. caninum* in dogs, in the States of Rio de Janeiro and Piauí. Dogs from areas endemic for Chagas disease in Piauí (municipality of São João do Piauí) and CVL-endemic areas in Rio de Janeiro (municipalities of Barra Mansa and Rio de Janeiro) were evaluated from March 2013 to December 2014.

For clinical examinations and the collection of biological samples including blood, intact skin fragments, lymph nodes, and bone marrow aspirates, dogs were sedated with an intramuscular injection of ketamine hydrochloride (10mg/kg) and acepromazine (0.2mg/kg). All samples were transferred to screw-cap tubes containing blood agar slants (Novy-MacNeal-Nicolle) overlaid with 1.5mL of Schneider's *Drosophila* medium (Sigma) supplemented with 10% fetal calf serum⁽⁵⁾. The culture-isolated strains were identified using nested PCR with specific oligonucleotides to amplify a partial region of the 18S rDNA gene. Nucleotide sequences were analyzed as previously described⁽⁶⁾. Six months later, an active search for infected dogs was performed with the aim of re-isolating parasites from skin fragments in the same region where the previous isolations were performed.

Nine samples of *Trypanosoma* sp. were isolated from nine dogs and analyzed in this study. The parasites were isolated exclusively from intact skin fragments. The dogs ranged in age from 3-5 years, and all were deemed to be asymptomatic and in good general health. Seven animals were from the municipality of Rio de Janeiro, one from the municipality of Barra Mansa, and another from the municipality of São João do Piauí (**Figure 1**). All isolates were identified as *T. caninum* and exhibited 100% homology with *T. caninum* sequences from previous studies⁽⁶⁾.

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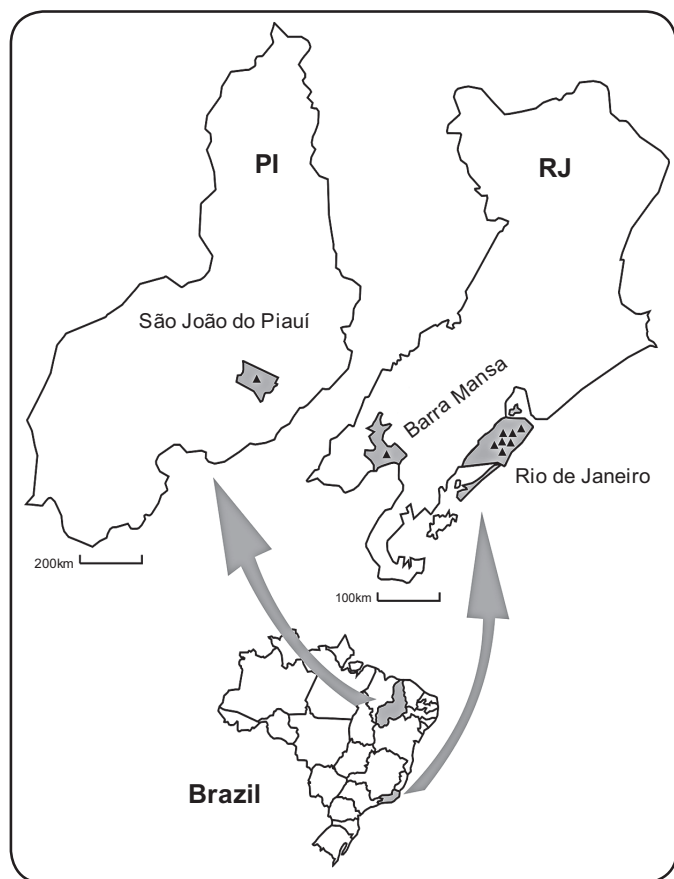


FIGURE 1 - Geographic locations of new cases of *Trypanosoma caninum* infection identified in the municipalities of São João do Piauí (PI), Barra Mansa, and Rio de Janeiro (RJ), in Brazil. PI: State of Piauí; RJ: State of Rio de Janeiro.

Six months after the initial isolation, four dogs previously diagnosed with *T. caninum* infection were found and re-evaluated. *T. caninum* was only re-isolated in one of these dogs; the remaining three were negative. The data on animals and samples processed for diagnostic purposes are summarized in **Table 1**, and the distribution of cases of *T. caninum* infection by municipality is shown in **Table 2**.

Trypanosoma caninum is a recently described species, and its occurrence has been reported in different States of Brazil⁽⁶⁾. Herein, we describe new cases of infection with this agent in two new transmission areas: Barra Mansa, in the State of Rio de Janeiro, and São João do Piauí, in the State of Piauí. All cases of *T. caninum* infection described to date have occurred in CVL-endemic areas, which highlights the need to assess the potential impact of the overlap of these species on CVL control⁽⁶⁾. Seven cases described in this study originated in the Atlantic forest region in the municipality of Rio de Janeiro, on the eastern slope of the Pedra Branca massif, in the lowlands in the Jacarepaguá district, where cases of CVL have been described since the 1980s⁽¹¹⁾. On the other hand, in Barra Mansa, a municipality located in the southern region of the State of Rio de Janeiro, CVL was only recently diagnosed⁽¹²⁾ and one of the cases described in this study came from this region.

The present study reports, for the first time, a case of *T. caninum* infection in dogs in the municipality of São João do Piauí, which is an endemic area for *T. cruzi*⁽¹³⁾. In areas where the endemic diseases overlap, diagnosis using serological tests is unfeasible because their specificity may not be sufficient. The geographical dispersion of *T. caninum* into areas in which other trypanosomatids (*i.e.*, *Leishmania* sp. and *T. cruzi*)

TABLE 1 - Diagnosis of *Trypanosoma caninum* in nine dogs determined by culture.

Dog number	Sample	Gender	Municipality/State	Culture			
				skin	bone marrow	lymph node	blood
1	A	M	Rio de Janeiro/RJ	+	-	-	-
1	B	M	Rio de Janeiro/RJ	-	ND	ND	ND
2	A	F	Rio de Janeiro/RJ	+	-	-	-
2	B	F	Rio de Janeiro/RJ	-	ND	ND	ND
3	A	M	Rio de Janeiro/RJ	+	ND	ND	ND
4	A	M	Rio de Janeiro/RJ	+	-	-	ND
4	B	M	Rio de Janeiro/RJ	+	ND	ND	ND
5	A	M	Rio de Janeiro/RJ	+	-	-	ND
5	B	M	Rio de Janeiro/RJ	-	ND	ND	ND
6	A	F	São João do Piauí/PI	+	-	ND	-
7	A	M	Barra Mansa/RJ	+	-	-	ND
8	A	M	Rio de Janeiro/RJ	+	-	ND	ND
9	A	F	Rio de Janeiro/RJ	+	-	-	ND

A: culture performed at baseline; B: culture performed after six months; M: male; F: female; +: positive culture; -: negative culture; ND: note done.

TABLE 2 - Cases of *Trypanosoma caninum* infection (as percentages) by each municipality studied.

Municipality (State)	Number	Percentage
Rio de Janeiro municipality (RJ)	103	6.8
Barra Mansa municipality (RJ)	234	0.4
São João do Piauí municipality (PI)	80	1.25

RJ: State of Rio de Janeiro; **PI:** State of Piauí.

circulate is a phenomenon that should be acknowledged and duly considered during diagnostic and epidemiological surveillance studies and interventions in these regions.

Our results are concordant with those previously published in the literature, in that to date *T. caninum* infection has only been found in intact skin fragments, as assessed by culture and PCR-based tests^{(7) (10)}. Moreover, the absence of clinical signs in the dogs studied, and the re-isolation of *T. caninum* in only one of the four dogs re-evaluated months later support the hypotheses that infection with this agent is asymptomatic, and can be transient⁽⁷⁾. The nine new cases described in this study, when added to those already published⁽⁶⁾, constitute a total of 62 cases of natural *T. caninum* infection reported in six states of Brazil. The identification of these new isolates, in addition to improving the knowledge-base with regard to the geographical distribution of this species in Brazil, is a warning sign for the relevant epidemiological surveillance sectors in the municipalities of Barra Mansa and São João do Piauí.

Ethical considerations

This study was approved by the Ethics Committee on Animal Experimentation of the Oswaldo Cruz Foundation [*Comissão de Ética no Uso de Animais do Instituto Oswaldo Cruz* (CEUA/FIOCRUZ), license LW-54/13].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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