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zacariascbpv@fcav.unesp.br

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Fernandes Leça Júnior, Nilo; dos Anjos Almeida, Valter; Santos Carvalho, Fábio; Rego
Albuquerque, George; Lessa Silva, Fabiana
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First report of *Trypanosoma cruzi* infection in naturally infected dogs from southern Bahia, Brazil

Primeiro relato de infecção natural por *Trypanosoma cruzi* em cães do sul da Bahia, Brasil

Nilo Fernandes Leça Júnior¹; Valter dos Anjos Almeida¹; Fábio Santos Carvalho¹;
George Rego Albuquerque²; Fabiana Lessa Silva^{2*}

¹Programa de Pós-graduação em Ciência Animal, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, Brasil

²Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, Brasil

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Abstract

In order to verify the *Trypanosoma cruzi* infection in domestic domiciled dogs in a rural endemic area from the south region of the State of Bahia, Polymerase Chain Reaction (PCR) were performed using S35 and S36 primers in 272 dogs living in the district of Vila Operaria, in the municipality of Buerarema. All animals were clinically evaluated; 2.5 mL of blood were collected through venipuncture for the performance of molecular tests. None of these animals showed clinical signs of the illness and only two were identified with the DNA parasite. This result is the first report of natural infection by *T. cruzi* in domestic dogs in southern Bahia.

Keywords: *Trypanosoma cruzi*, dog, PCR, Polymerase Chain Reaction, Bahia.

Resumo

Com o objetivo de verificar a infecção por *Trypanosoma cruzi* em cães domésticos domiciliados em área rural e endêmica do sul da Bahia, foi realizada a Reação em Cadeia da Polimerase (PCR), utilizando-se os iniciadores S35 e S36 em 272 cães domiciliados no distrito da Vila Operária, cidade de Buerarema. Todos os animais foram avaliados clinicamente e, posteriormente, foram coletados 2,5 mL de sangue por punção venosa para realização do diagnóstico molecular. Nenhum dos animais apresentou manifestação clínica da doença e, em apenas dois foram identificados DNA do parasito. Esse resultado é o primeiro relato de infecção natural por *T. cruzi* em cães domésticos no sul baiano.

Palavras-chave: *Trypanosoma cruzi*, cão, PCR, Reação em Cadeia da Polimerase, Bahia.

Introduction

American trypanosomiasis is a major public health problem in developing countries. This disease is caused by a protozoan parasite that can affect both human and wild or domestic animals. In Brazil, according to Vinhaes and Dias (2000), 36% of the whole territory presents risks of vectorial transmission. In the northeast region, the State of Bahia holds the highest level of human infection, with seroprevalence rates varying from 5.4 to 25.0% (CAMARGO et al., 1984; ARAS et al., 2002). The main form of contamination is by the feces of contaminated hematophagous triatomine arthropods (ROSPAL et al., 2007) however, contamination through experimental breast-feeding (MEDINA LOPES, 1988), blood transfusion (BAHIA et al., 2002), and oral ingestion (YOSHIDA, 2009; SHIKANAI-YASUDA; CARVALHO, 2012) have been reported. Sylvatic and domestic animals play an important role in the life cycle of this parasite.

Domestic dogs act maintaining the lifecycle of this parasite in the ambience, especially inside their dwellings, because those animals are a frequent source of blood meals for triatomines bugs (GÜRTLER et al., 1997, 2007). In the State of Bahia, previous studies carried out in rural areas verified approximately 19.0% of dogs infected by *T. cruzi* - detected by xenodiagnosis (MOTT et al., 1978; BARRET et al., 1979). The main clinical signs observed in sick animals are anorexia, fever, lymphadenopathy and problems related to heart failure (NABITY et al., 2006; GUEDES et al., 2007). The resource tools commonly used to diagnose this disease are the parasitological, immunological and molecular tests. The purpose of the present research was to identify *Trypanosoma cruzi* infection in dogs in an endemic rural area of southern Bahia, a district with no data of barber bug fever (Chagas disease) in dogs.

Materials and Methods

The district of Vila Operaria in the municipality of Buerarema (14° 57' S and 39° 19' W), south region of the State of Bahia, Brazil, is an endemic area for American trypanosomiasis. The

*Corresponding author: Fabiana Lessa Silva

Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz – UESC, Rod. Ilhéus Itabuna, Km 16, Salobrinho, CEP 45662-900, Ilhéus, BA, Brasil

e-mail: fabiana.lessa@gmail.com

district is surrounded by the native Atlantic Rainforest and the site of this study presents a strictly rural structure with its main economic resources coming from cocoa farms.

In this study, the whole dog population living in the district was included and all the animals were natural of the area. From the total of 272 animals, 158 (58.08%) were males and 114 (41.92%) were females. Initially, the animals were clinically evaluated, then 2.5 mL of blood was obtained through venipuncture and allocated in Vacutainer® tubes with EDTA in a cooler box to perform molecular tests.

The materials were centrifuged for 15 minutes at 1473.35 G and the leucocitary layers were transferred to micro tubes free of DNAase and RNAase and stored at -20 °C until the procedure of DNA extraction.

DNA extraction was performed utilizing phenol-chloroform method as a routine protocol of the Laboratory of Animal Genetics – Veterinary Medicine Hospital – UESC. The material obtained from this procedure was stocked at -20 °C until PCR diagnostic test.

PCR amplification was performed in a total volume of 25 µL containing 0.4 µL (1.5 U) of Taq DNA Polymerase (Invitrogen®), 17 µL of Supermix (Invitrogen®), 0.6 µL (1.2 mM) of MgCl₂, 2 µL (20 picomol) of each primer, and 3 µL of the DNA extract. The sequences of primers used in the PCR reaction were S35: 5' AAATAATGTACGGGGGAGATGCATGA 3' and S36: 5' GGGTTCGATTGGGGTTGGTGT 3'. Those primers amplified a fragment of 330 pb. The reaction conditions, such as number of cycles and temperature, were adapted from Ávila et al. (1990). The reactions were performed utilizing 35 cycles and the following temperature profile: denaturation at 94 °C for 1 minute (initializing with a maximum period of 5 minutes at 94 °C), 65 °C for 1 minute for primer annealing and 72 °C for 1 minute for extension, and final incubation for 7 minutes at 72 °C. The amplified DNA was visualized with 2% agarose gel, revealed with bromide ethidium (10 mg/mL), and photo documented. All PCR reactions were compared to positive and negative controls. The positive controls were obtained from pure culture of *T. cruzi* Y strain and ultra-pure water was used for the negative control.

This study is consistent with ethical principles of animal experimentation. It was approved and authorized by the Animal Ethic Committee from the "Universidade Estadual de Santa Cruz" – UESC, (Protocol nº. 002/10).

Results and Discussion

From the 272 dogs studied, only two (0.7%) animals, one male and one female, were positive in the molecular diagnosis for DNA from *T. cruzi* parasite (Figure 1).

At the clinical exam, no sign of the disease was observed in the dog population. Machado et al. (2001) observed dogs that did not show clinical evidence of the disease even after five episodes of reinfection and dogs that presented only sporadic febrile state during the first weeks after each inoculation.

The available data on the incidence of *T. cruzi* infection in dogs are based on immunological tests, once there are no epidemiological studies based on PCR. In Brazil, data from dogs in the State of

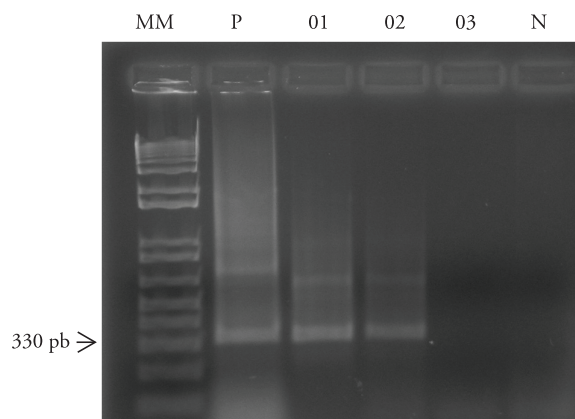


Figure 1. Representative result of the PCR for detection of *T. cruzi* in blood samples of dogs living in the district of Vila Operária, city of Buerarema. MM = Molecular Marker; P = Positive Control (culture of *T. cruzi* – Y strain); 01 to 03: dog samples; N = Negative control (ultra-pure water). Observe band presence in the animals 01 and 02.

Mato Grosso do Sul, an endemic autochthone area for human Chagas disease, showed 45.3% and 24.0% of infection in domiciled animals with IFI (indirect immunofluorescence test) and ELISA (enzyme-linked immunosorbent assay), respectively (SOUZA et al., 2009). However, in the State of Sao Paulo, 107 serum samples from euthanized dogs were tested by IFI, revealing no positive titers for *T. cruzi* infection (ROSYPAL et al., 2007). This wide range observed in the results obtained by immunological tests that have high sensibility and low specificity can be explained by previous related cross reaction, in humans and dogs, between *Leishmania* spp. and *T. cruzi* infections (LUCIANO et al., 2009).

Molecular tests such as PCR provide a good alternative tool for detection of *T. cruzi* infection in animal samples. The values observed with this technique, in experimental studies with dogs, reached more than 90% using blood or others tissues (ARAÚJO et al., 2002; VELOSO et al., 2008). Veloso et al. (2008) obtained the highest levels in identifying parasitized experimentally infected Beagle dogs that were in the acute phase of infection, when the parasitemia levels were high. The low incidence observed in this study can be explained by the fact that those animals were possibly on the chronic phase of the infection or were not really infected.

In Brazil, since the 1990's, the Brazilian National Health Foundation (FUNASA) has enforced intensive control measures on the *Triatoma infestans* population through insecticide spraying. The result of this control program was the widespread reduction of *T. cruzi* transmission by this triatomine bug, considered the most important vector of Chagas disease in the past (SILVEIRA; VINHAES, 1999; DIAS, 2006). In 2006, the Pan American Health Organization (PAHO) certified the interruption of *T. cruzi* transmission by *T. infestans* in Brazil (BRASIL, 2006). Nevertheless, according to the Brazilian Health Minister (BRASIL, 2005), the State of Bahia is the only one in the country that still presents infestation by *T. infestans*, with 93 of the 417 municipalities situated in risk areas or presenting the vectors over the past years. Moreover, there are reports that suggest the participation of triatomine bugs, otherwise considered sylvatic, in the transmission of the disease

in that state, for instance *T. pseudomaculata*, *T. tibiamaculata* and *T. brasiliensis* (ALMEIDA et al., 2009; DIAS-LIMA; SHERLOCK, 2000; WALTER et al., 2005; SANTANA et al., 2011). Besides, this data suggest the maintenance of vectorial importance in *T. cruzi* transmission in that region. Thus, in spite of the low infection prevalence observed in this research, these data are important, once infected dogs can act as *T. cruzi* reservoirs.

This is the first report of infected dogs in southern Bahia. It is an important finding because it suggests that those dogs can act in the life cycle of *T. cruzi* parasite in an endemic area for the disease, being a risk factor to contaminate triatomine bugs and then humans.

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