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Trypanosoma cruzi INFECTION IN NINE-BANDED ARMADILLOS FROM ESPÍRITO SANTO STATE, BRAZIL

INFECÇÃO POR *Trypanasoma cruzi* EM TATUS-GALINHA NO ESTADO DO ESPÍRITO SANTO, BRASIL

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RESUMO

Um total de 61 tatus-galinha (*Dasypus novemcinctus*) foram testados para infecção por *Trypanosoma cruzi*; o agente causador da doença de Chagas ou tripanossomíase americana. A prevalência por meio da Reação em Cadeia da Polimerase (PCR) foi de 6,55%. Estes resultados sugerem que o tatu pode ser um possível reservatório desta zoonose no Estado do Espírito Santo, Brasil.

Palavras-chave: Brasil, doença de Chagas, *Dasypus novemcinctus*, *Trypanosoma cruzi.*

SUMMARY

A total of 61 nine-banded armadillos (*Dasypus novemcinctus*) were tested for *Trypanosoma cruzi* infection; the causative agent of Chagas disease or American trypanosomiasis. The prevalence through Polymerase Chain Reaction (PCR) was the 6.55%. These results suggest that the armadillo may is a possible reservoir of this zoonosis in the state of Espírito Santo, Brazil.

Keywords: Brazil, Chagas diseases, *Dasypus novemcinctus*, *Trypanosoma cruzi*.

INTRODUCTION

American trypanosomiasis, or Chagas disease (CD) was first described in 1909 by the Brazilian Carlos Chagas; being the most important parasitic infection in Latin America. More than 10 million people carry the protozoan agent, *Trypanosoma cruzi*, transmitted by triatomine bugs (Miles *et al.*, 2003). Over 200 species/subspecies of mammals and 120 triatomine species are known to be susceptible to infection by *T. cruzi* (Dias, 2000). The disease is a complex zoonosis, with mammals as a natural reservoir hosts (Miles *et al.*, 2003). CD occurs in nature as a sylvatic cycle, where *T. cruzi* interacts with wild triatomines and mammalian reservoirs, such as marsupials, rodents and armadillos (Fernandes *et al.*, 1999). Armadillos from *Dasypus spp*. were the first silvatic host of *T. cruzi* to be described, by Carlos Chagas in Brazil (Chagas, 1912). In South America nine-banded armadillos (*Dasypus*

novemcinctus) have been found to be infected in Venezuela (Torrealba, 1937), Panama (Clark and Dunn, 1932), México (Brumpt *et al.,* 1939), and in Paraguay (Yeo *et al.*, 2005). Lainson *et al.* (1979) described nine-banded armadillos infected in State of Pará, Brazil, and Fernandes *et al.* (1999) did not found armadillos infected in the State of Rio de Janeiro, Brazil. In United States, nine-banded armadillos are considered natural reservoir of CD (Paige *et al.*, 2002). Little is known about the epidemiology of CD in armadillos or the role that they may act in human infections (Herwaldt *et al.*, 2000). The present study was aimed at evaluating the prevalence of free-ranging nine-banded armadillos to Chagas diseases.

MATERIALS AND METHODS

The Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) has authorized the capture and handling of the armadillos. The Ethics Committee on Animal Experimentation of the University Federal of Espírito Santo, Vitória, Brazil, approved the project. During a research of *Mycobacterium leprae* in armadillos from Espirito Santo state, spleens of 61 animals were tested for the possibility to act with reservoirs of CD. Armadillos were collected from July 2005 to July 2006 in the state of Espírito Santo, Brazil (Fig. 1). Armadillos were captured by traps and anesthetized (Fournier-Chambrillon *et al.*, 2000). After physical examination the armadillos were killed under anesthesia by exsanguinations and a thorough autopsy was done (Walsh *et al.*, 1975). Spleens were collected and stored at -20ºC for Polymerase Chain Reaction (PCR). The 195-basepair-satellite repeat of nDNA is the target of the primers Tcz1/Tcz2 (Moser *et al.*, 1989), which not amplify DNA from others Trypanosomas or Leishmanias. The samples were processed for DNA extraction using the DNeasy® Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The primers used for the amplification of *T. cruzi* were Tcz1 (CGAGCTCTTGCCCACACGGGTGCT), and Tcz2 (CCTCCAAGCAGCGGATAGTTCAGG). PCR protocol including AmpliTAQ Gold® polymerase (Applied Biosystems, Foster City, CA) in a 50 µL reaction volume: DNA template (50-200 ng), 25.75 µL of dH2O, 5 µL of 10X PCR reaction buffer, 5 µL of MgCl2 (2µM), 4 µL of deoxynucleotide triphosphate mix (10 µM), 2.5 µL of each primer (20 µM), and 0.25 µL of AmpliTAQ Gold®. The cycling program included an initial denaturation at 95^0C for 5 minutes, 40 amplification cycles at 95^0C for 15 seconds, 60^0C for

15 seconds and 72^{0}C for 30 seconds, and a terminal extension at 72^{0}C for 7 minutes. Negative controls (dH_2O) were included in each PCR. A 17 µL aliquot of each PCR product was then added to the wells of a 2% agarose gel containing ethidium bromide (E-gel®, Invitrogen, Carlsbad, CA), and electrophoresed for 30 minutes at a constant voltage. The PCR fragments were visualized by UV transillumination, and documented using the Gel Doc 2000 (Bio-Rad, Hercules, CA).

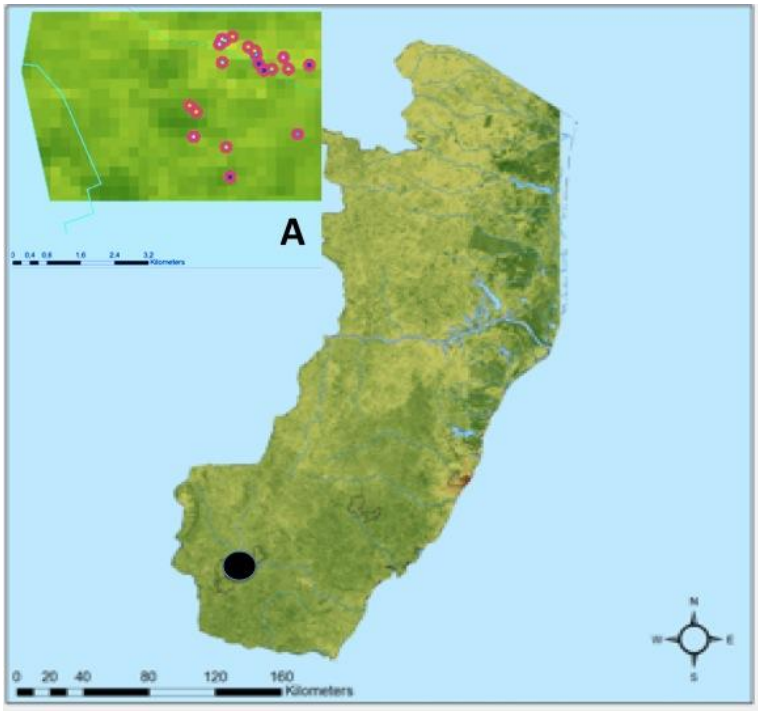

Figure 1- Map 1- Main map of Espirito Santo State, Brazil, showing the areas where the armadillos were captured (demarcated circled area in black). **A -** Zoom from the area where the traps were used to capture the armadillos.

RESULTS

Four (6,55%) armadillos were positive to *T. cruzi* in PCR technique (Fig. 2).

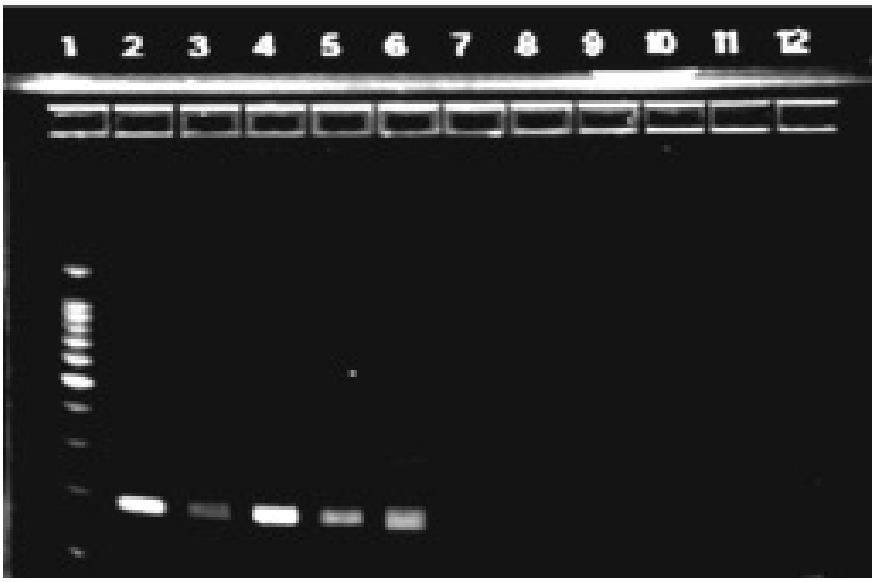

Figure 2 - Image of 2% agarose gel demonstrating amplification of DNA (188bp) from *Trypanossoma cruzi.* Lane 1: Ladder 100bp; Lane 2-5: positives armadillos; Lane 6: positive control; Lane 7: negative control for DNA extraction; Lane 8: negative control for PCR; Lane 9-12: negative samples.

DISCUSSION

Similar *T. cruzi* infection rates were described by others authors (Paige *et al.,* 2002). The results demonstrated that the PCR protocol (Moser *et al.,* 1989) achieved for diagnosis of CD in humans can be used for the diagnosis of CD in a possible free-ranging natural reservoir. The autochthonous transmission of *T. cruzi* in the United States is associated with armadillo's population that hosts *T. cruzi* (Dorn *et al.,* 2007). Several authors have indicated free-ranging mammals are increasingly exposed to *T. cruzi* (Diaz, 2007). Recent reports documented that domestic animals, particularly outdoor dogs, are infected by triatomines possessing wild animal strains of *T. cruzi* (Diaz, 2007). The requirements for autochthonous Chagas' disease transmission include a competent reduviid vectors for *T. cruzi*, free-ranging and domestic animal reservoirs of *T. cruzi*, and susceptible human hosts (Rosypal *et al.*, 2011). At the state of Espírito Santo there is no study about the prevalence of Chagas Disease in humans. In conclusion, the *D. novemcinctus* are considered natural reservoir of CD in others countries and, the finding of positive armadillos for *T.*

cruzi suggests that nine-banded armadillos also may act as a possible reservoir of this zoonosis (CD) in the state of Espírito Santo, Brazil.

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