

## Short Communication

# Survey of *Trypanosoma* (Kinetoplastida: Trypanosomatidae) Infection in Monte Negro Municipality, State of Rondônia, Western Amazon, with First Record of *T. evansi* in the state.

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### Abstract

**Introduction:** Trypanosomes can infect humans and animals. This is the first record of the occurrence of *Trypanosoma evansi* in Rondônia. **Methods:** Blood samples were collected from 7 dogs and 22 humans. Furthermore, triatomines and tabanids were collected. **Results:** It was observed that 42.8% of the dogs tested positive for *T. evansi* and 14.3% presented mixed infection; 15% of the triatomines tested positive for flagellates identified as *T. cruzi* TCI (3 specimens), *T. cruzi* TCI, and *T. rangeli* (1 specimen), and one with *T. cruzi* TCV. Two tabanids were infected with *T. theileri*. **Conclusions:** These findings may benefit vector control strategies.

**Keywords:** *Trypanosoma cruzi*. *Trypanosoma evansi*. *Trypanosoma theileri*. Chagas disease. Surra. Amazon.

*Trypanosoma* is a protozoan genus that causes a range of infections in the most varied hosts<sup>1,2</sup>. These flagellates transmit disease by the bite of infected insect vectors, mechanical transmission, contact with vector feces and/or urine, or ingestion of infected meat<sup>1,2,3</sup>.

There are trypanosomes of medical importance, such as *Trypanosoma cruzi*, the etiological agent of Chagas disease, and of veterinary importance, such as *Trypanosoma evansi*, the etiological agent of Surra disease or “Mal das Cadeiras,” an animal trypanosomiasis that affects a variety of economically

important mammals, such as horses, buffaloes, goats, sheep, pigs, camels, and wild animals (coati and capybara), as well as dogs, cats, and even humans<sup>1,2,4,5</sup>. *T. evansi* is transmitted mechanically and through the bites of haematophagous flies, haematophagous bats<sup>2,4,6,7</sup>. *T. theileri* is often considered to be nonpathogenic and contains some genera of tabanids as vectors<sup>8</sup>. However, clinical signs such as anemia and abortion have been described in cattle populations<sup>9</sup>.

By ingesting raw meat from infected wild mammals, dogs can become infected with *T. cruzi* and *T. evansi*<sup>2,7</sup>. Following infection by *T. evansi*, they typically exhibit weakness, fever, anemia, edema, uveitis, hepatomegaly, splenomegaly, and paraplegia, which lead to death<sup>2,7</sup>.

This study aimed to report the first occurrence of *T. evansi* in Rondônia, in the Western Brazilian Amazon.

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**Received** 02 June 2019

**Accepted** 02 August 2019

The investigation was performed during a public health control action conducted in the rural region of the Monte Negro municipality, Rondônia, Brazil (**Figure 1A**). On 28 June 2018, research was conducted on an alleged outbreak of Chagas disease at the Santo Antônio Farm on Route C 20 in the rural area of Monte Negro, 18 km from the municipal offices (10°15'07.62"S 63°19'51.24"W (**Figure 1B**)).

At the time, the team from the Institute of Biomedical Sciences 5 - (ICB-5/USP) visited the location at the request of the Municipality Health Secretary (MHS) and performed physical examinations on dogs and humans, collected material for laboratory examinations from local dogs, and captured triatomines and tabanids.

During the visit, 22 local inhabitants were examined physically in addition, one of the 22 patients was subjected to a digital electrocardiogram (DECG). Blood samples were collected for optical microscopy, indirect immunofluorescence, and ELISA (IgM) testing for Chagas disease. The ICB-5/USP staff collected blood samples from seven dogs and subsequently captured insect vectors. Thirty-three triatomines (to search for presence of trypanosomatids in the gastrointestinal tract) and five tabanids were captured. The triatomines were captured in four specimens of *Attalea* sp. (babassu palm) located near the residence, in which their bracts were removed using a chainsaw. The tabanids were collected using three NZI traps assembled in the residential area over 2 days, a 1 m × 2 m Malaise trap for a total of 8h and two catchers in a 5h capture effort directly from the horses.

Blood samples from the dogs were preserved in ethanol P.A. 70% and sent to the ICB-2/USP in São Paulo for molecular analysis (parasite DNA amplification and DNA sequencing). The identification of trypanosomes was performed using the fluorescent fragment length barcoding method, as described by Hamilton et al. (2008)<sup>10</sup> and adapted for South American trypanosomes by Hamilton et al. (2011)<sup>11</sup> and Garcia et al. (2018)<sup>12</sup>. The technique involves species diagnosis by the profiles yielded through polymerase chain reaction (PCR) amplification of two segments, each of the 18S and 28S units of the ribosomal DNA. The primers used for the amplification of the four segments were as follows:

18S-1f ACCGWTTTCGGCTTTTGTGG

18S-2r (bluea) CGGTCTAAGAATTTACCTC

18S-3 GACCRTTGTAGTCCACACTG

18S-4r (green) CCCCTGAGACTGTAACCTC

28S-1f (blue<sup>a</sup>) GAAAGAGAGTGACATAGAAC

28S-2r TGTTTCAAGACGGGTGGGGGC

28S-2f (black<sup>a</sup>) CCCCCACCCGTCTTGAACA

28S-3r GGGTCCAAACAGGCACACTC

The profiles produced by the four fluorescent peaks resulting from the PCR amplification were determined on an ABI 3500

sequencer and further analyzed using the GeneMapper Software v.4.0 (Applied Biosystems). Each trypanosome species yields an exclusive and diagnostic fragment length profile. Novel profiles from unknown trypanosome species or lineages were identified by gGAPDH and SSU rRNA sequencing.

The triatomines were morphologically identified in accordance with Jurberg et al. (2014)<sup>13</sup>. To determine the percentage of specimens that tested positive for trypanosomatids, fecal material obtained by abdominal compression was examined. The specimens that tested positive for trypanosomatids were subsequently subjected to intestinal extraction and the intestines were preserved in ethanol P.A. 70% for further molecular analysis. To identify the trypanosomatid species, standardized diagnostic PCR was used<sup>14</sup>.

The captured tabanid specimens were morphologically identified by the eighth author, a tabanid specialist, and compared with the specimens identified from the entomological collection of the National Institute of Amazonian Research.

The research project was submitted and subsequently approved by the Animal Ethics Committee from the Instituto Oswaldo Cruz Foundation -Rondônia - CEUA-FioCruz n<sup>o</sup> 2019/06.

The specimens were collected with permission from the Brazilian Institute of Environment and Renewable Natural Resources [Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)], permanent license Nr. 52260-1.

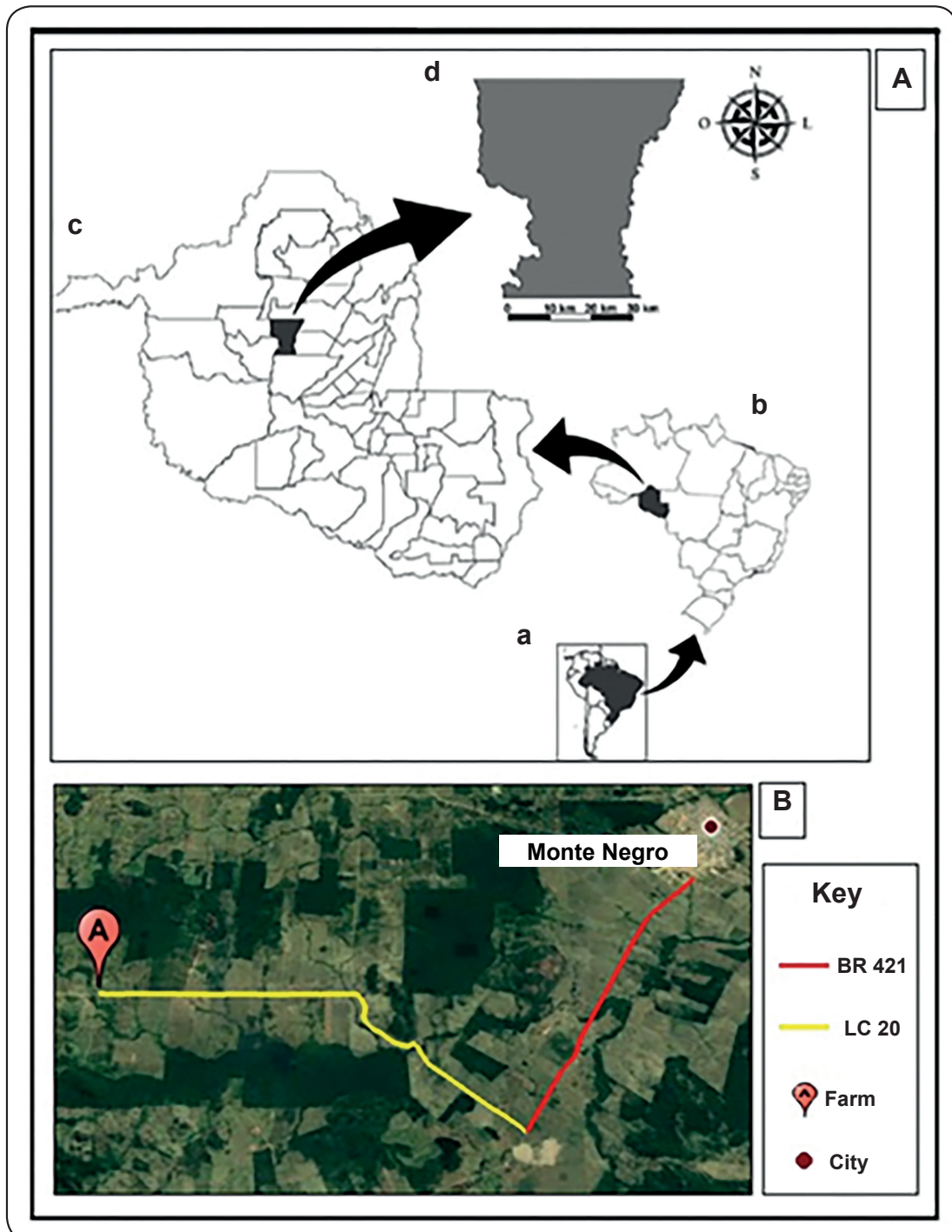
The DECG examination of a 76-year-old patient from Itapipoca, Ceará, Brazil, presented alterations compatible with the right bundle branch block of the cardiac conduction system and 2 extrasystoles/min (plausible for chronic Chagas disease).

All the 22 human blood samples collected by the MHS were examined microscopically (1.000x) and were tested using indirect immunofluorescence and ELISA techniques. After examination, all the 22 blood samples tested negative (secondary data), including that of the patient with DECG alterations and systemic arterial hypertension.

Among the seven dogs examined, three presented positive results for the examination using molecular techniques, two for *T. evansi*, and one for co-infection by *T. evansi* and *T. cruzi*. The three dogs eventually succumbed to death 2 to 3 months following collection.

The 33 triatomines collected were identified as *Rhodnius montenegrensis*. Seven of them were adults, eight were nymphs in the fifth stage, 10 were nymphs in the fourth stage, five were nymphs in the third stage, and three were nymphs in the second stage. Among these, five (15%) tested positive for trypanosomatids through a fresh examination of fecal material. By molecular analysis, trypanosomatids were identified as belonging to the species *T. cruzi* TCI (three specimens), *T. cruzi* TCI (one specimen), *T. rangeli* (one specimen), and *T. cruzi* TCV (one specimen).

The five tabanid specimens captured were identified as *Chrysops laetus* Fabricius, 1805 (one female), *Chrysops varians* Wiedemann, 1828 (one female), and *Dichelacera tetradelta* Henriques, 1995 (three females), of which two were infected



**FIGURE 1:** (A): Map of the Monte Negro municipality, Rondônia. (a): Brazil, (b): State of Rondônia, (c): State of Rondônia with the municipality of Monte Negro highlighted, (d): Municipality of Monte Negro. (B): Site of the study in relation to the municipal offices of Monte Negro.

with *T. theileri* (*C. laetus* and *D. tetradelta*) and one with *T. vivax* (not included). Heavy rain in the period reduced the amount of insects caught.

Even though we did not obtain positive results in the hemoscopy, indirect immunofluorescence, and ELISA examinations of the human blood, a positive result found for *T. cruzi* in one dog underlining the occurrence of the etiological agent of Chagas disease at the location indicates a need for

surveillance actions. These animals are considered sentinels in *T. cruzi* transmission<sup>15</sup> and the occurrence of *T. cruzi* infection in the dog may be related to the presence of infected vectors near the residence<sup>3</sup>. Meanwhile, Bilheiro et al. (2018) found triatomines of the species *R. montenegrensis* infected with *T. cruzi* in the vicinity of rural residences within the municipality<sup>16</sup>.

Three of the seven dogs analyzed (42.8%) were infected with *T. evansi*. Previous studies have shown that dogs are mammals

that are highly susceptible to *T. evansi* and the infection of these animals results in high parasitemia, favoring transmission<sup>7</sup>.

Although the capture effort was modest and hampered by rainfall, the captured tabanid specimens of the genus *Chrysops* have already been described with vector potential for *T. evansi*<sup>6</sup>. The implementation of future capture efforts and at different times of the year will be performed to expand upon this context.

Following confirmation of trypanosomiasis at the location, the population was instructed to ensure that dogs are sent to the veterinarian for treatment. Local residents were also instructed to capture and send any triatomine-like insects found in the residential or surrounding areas to the ICB5-USP.

The transmission of *T. evansi* in rural areas, previously unknown in the region, may cause losses with economic impacts on local populations, as it can affect different species, such as horses, cattle, pigs, sheep, goats, and buffaloes<sup>1,2,6</sup>.

Radwanska et al. described the infection of tabanids by *T. vivax*. This parasite, which is transmitted by some genera of haematophagous flies, affects cattle and buffaloes, causing acute and chronic febrile consumptive illnesses, with significant economic loss arising from the death and/or weakening of the herds<sup>1</sup>.

The data obtained in this study demonstrate the need for more comprehensive studies to verify the occurrence of *T. vivax* in both dogs and other mammalian species. Furthermore, it is necessary to expand upon the analyses to identify haematophagous flies, i.e., “horse flies that may actively transmit *T. evansi* and *T. theileri* in this region, such that prophylaxis and control strategies can be directed.

The occurrence of *T. cruzi* infection in dogs is a risk factor associated with the active transmission of human Chagas disease. Consequently, the training of public health service microscopists, who had performed the diagnosis of leishmaniasis and malaria, is highly important for the diagnosis of trypanosomes. In addition, educating veterinary professionals about the occurrence of parasites in the region is crucial.

## ACKNOWLEDGMENTS

We thank Prof. Erney Plessmann Camargo (INCT-EpiAmo) for the molecular identifications of the trypanosomatides; Prof. Marcelo Bahia Labruna (FMVZ/USP) and Prof. Dionatas Meneguetti (UFAC) for the review of the manuscript; Carla M. Rodrigues (INCT-EpiAmo) for the molecular identifications of the trypanosomatides; and Willian Rocha for support in the capture of triatomines.

## Financial Support

The study was supported by the National Institute of Science and Technology from the Brazilian Centre of Science and Technology-CNPq/EpiAmo and the Research Foundation of the State of São Paulo (FAPESP).

## Conflict of Interest

The authors declare that they have no conflict of interest.

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