Rev Soc Bras Med Trop 50(6):829-832, November-December, 2017
doi: 10.1590/0037-8682-0054-2017

Short Communication

Rhodnius stali: new vector infected by *Trypanosoma rangeli* (Kinetoplastida, Trypanosomatidae)

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Abstract

Introduction: *Rhodnius stali* infection by *Trypanosoma rangeli* is reported in this study for the first time. **Methods**: The triatomines were collected from the campus of the Federal University of Acre in Rio Branco, Acre, Brazil. The identification of *T. rangeli* was confirmed by multiplex polymerase chain reaction. **Results**: The examinations of two specimens revealed *R. stali* infection by the epimastigote forms of *T. rangeli*. **Conclusions**: The encounter of *R. stali* infected by *T. rangeli* generates an alert for the state of Acre, since the simultaneous presence with *Trypanosoma cruzi* can make the differential diagnosis of Chagas disease difficult.

Keywords: Triatomines. Rangeliose. Trypanosomatids.

The protozoan *Trypanosoma rangeli* is a hemoflagellate parasite, belonging to the family Trypanosomatidae, and it generally infects invertebrate hosts, such as hematophagous insects, and vertebrate hosts such as mammals, including humans. Its transmission occurs mainly during the blood feeding of some species of triatomines[1].

Human infections such as those caused by parasites have been reported in Central and South America, including Brazil, where cases of human rangeliosis have been reported in the States of Amazonas, Pará, Alagoas, Minas Gerais, Santa Catarina, and Bahia[2,3].

Although there have been no reports of adverse health effects caused by *T. rangeli* in vertebrates, this protozoan is considered to be pathogenic to invertebrates[1].

It is recognized that the species of triatominae belonging to the genus *Rhodnius* are susceptible to infection by *Trypanosoma rangeli*, and the occurrence has already been recorded in the following species: *Rhodnius domesticus*, *Rhodnius nasutus*, *Rhodnius neglectus*[4], *Rhodnius pallescens*, *Rhodnius prolixus*, *Rhodnius robustus*[5], *Rhodnius brethesi*[6], *Rhodnius colombiensis*, *Rhodnius ecuadoriensis*[7], *Rhodnius dalessandroi*, *Rhodnius pictipes*[8], *Rhodnius montenegrensis*[9], and *Rhodnius neivai*[10]. However, there are no records of *T. rangeli* infection in *Rhodnius amazonicus, Rhodnius barretti, Rhodnius milesi, Rhodnius paraenses,* and *Rhodnius zeledoni*, also considered to be possible vectors of this parasite[8].

This study describes the first report of *R. stali* infected by *T. rangeli*. Two specimens of *R. stali* (**Figure 1**) were collected on the campus of the Federal University of Acre [*Universidade Federal do Acre* (UFAC)] in the City of Rio Branco, Acre, Brazil (Lat. 9º57′12″S, Long. 65º″51′.48″W) (**Figure 2**), probably attracted by the campus lighting. The triatomines were found in the vicinity of the Zoobotanical Park at the University, a location that contains several palm trees of the genus *Attalea*, which are considered to be natural ecotones for *R. stali* in the southwest region of the Amazon[11]. This locality also contains wild mammals such as bats, agoutis, and capybaras that circulate frequently in the dependences of the university.

Identification of the triatomines was carried out in the Department of Biological Sciences of the Faculty of Pharmaceutical Sciences, *Universidade Estadual Paulista*

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Received 9 February 2017
Accepted 24 August 2017

FIGURE 1 - Dorsal view of *Rhodnius stali.*

Júlio de Mesquita Filho (UNESP), Araraquara, São Paulo, Brazil, via comparison between characteristics of *R. stali* genitalia and those of *R. pictipes* from the same insectarium (CTA 71), collected in Belém, Pará. These characteristics have been reported as being similar to those described by Lent et al.[12,13].

Initially, fresh and stained smears were prepared with 0.1% triarylmethane, 0.1% xanthenes and 0.1% thiazines) from the contents of the triatomine rectal ampulla and were then analyzed under a 1,600X optical microscope (**Figure 3A and B**).

The trypanosomatid species were confirmed by multiplex polymerase chain reaction (PCR). Parasite deoxyribonucleic acid (DNA) was extracted from the triatomine rectal samples using a Qiagen DNA extraction kit®. The multiplex PCR was performed according to a protocol described by Fernandes et al.[14]. This method amplifies a portion of the non-transcribed spacer of the mini-exon gene that varies between *T. cruzi* and *T. rangeli* species, and between lines 1 and 2 of *T. cruzi*. The following primers were used: TC1, 5'-ACACTTTCTGGCGCTGATCG-3'; TC2, 250 bp, 5'-TTGCTCGCACACTCGGCTGCAT-3'; Z3, 150 bp, 5'-CCGCGCACAACCCCTATAAAAATG-3'; TR, 100 bp, 5'-CCTATTGTGATCCCCATCTTCG-3' and EXON, 5'-TACCAATATAGTACAGAACTG-3'. The reaction mixture consisted of 100pmol of each primer and 150µM deoxynucleotide triphosphates (dNTPs) in a buffer composed of 10mM Tris-HCl (pH 8.3), 1.5mM MgCl2, 25mM KCl, 0.1mg/mL bovine serum albumin, 2.5U of Taq DNA polymerase,

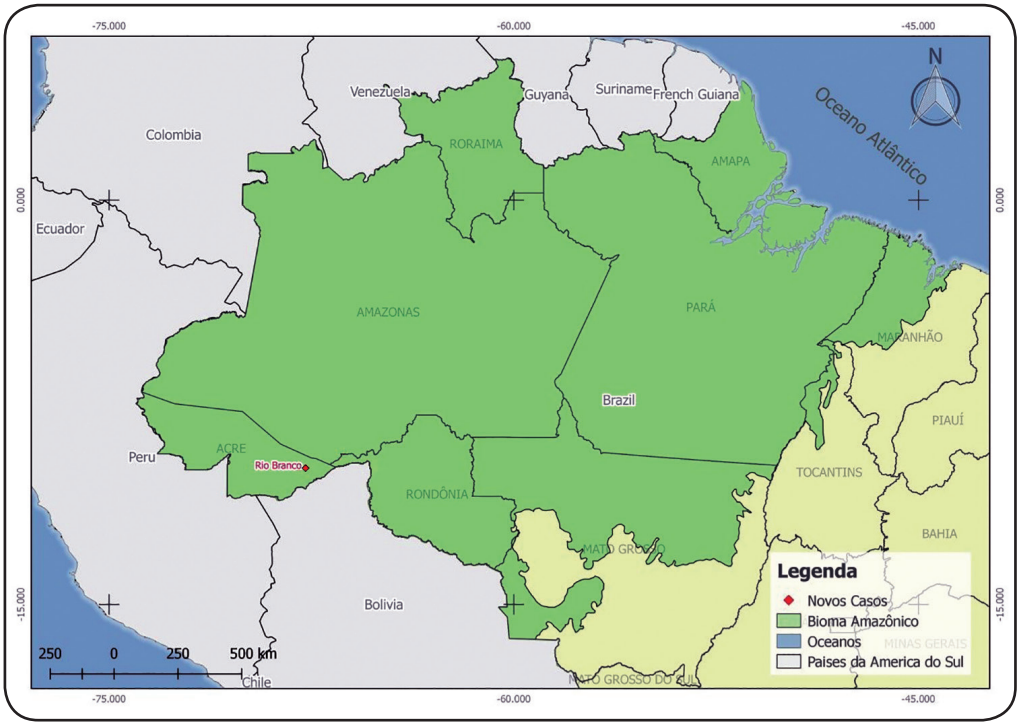

FIGURE 2 - Geographical location of the municipality of Rio Branco, State of Acre, Brazil.

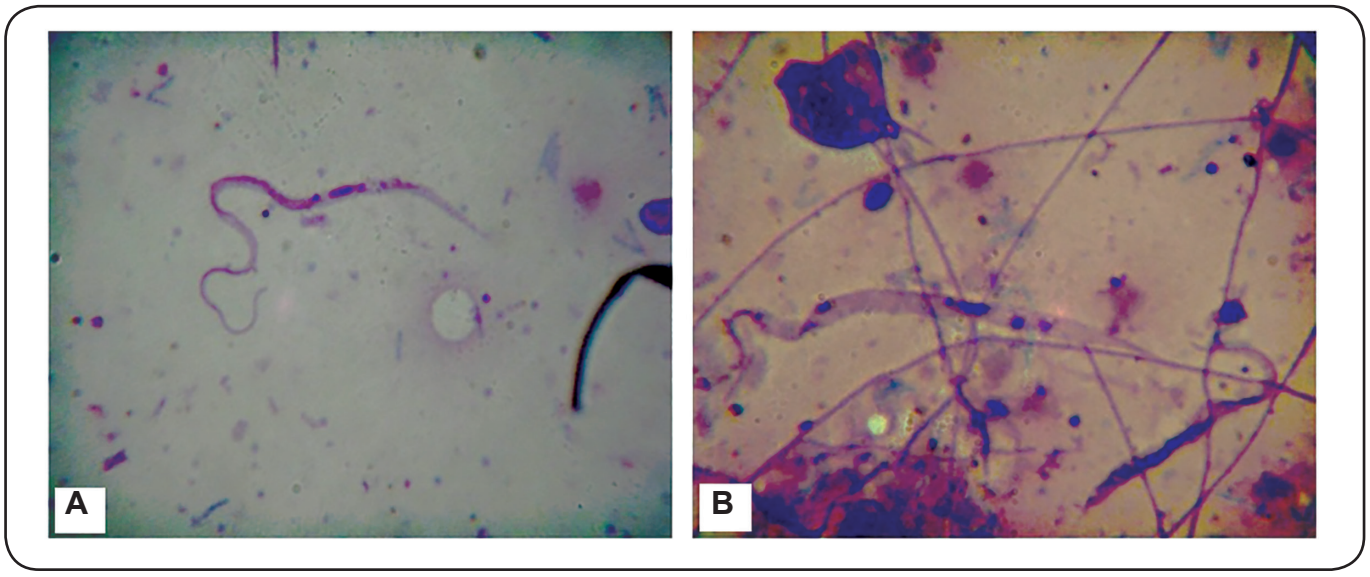

FIGURE 3 - A and B: Epimastigote form of *Trypanosoma rangeli* at 1,600X magnification.

and 10ng of genomic DNA in a total volume of 50µL. The thermal cycling conditions were as follows: an initial step of 5 min at 95°C, 34 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, and a final extension of 10 min at 72°C. The following reference strains were used as controls in each reaction: TC1, X10 Clone 1; TC2, Strain Y; Z3, Emerald Clone 1, and *T. rangeli* R1625. The amplified products were subjected to electrophoresis on a 2% agarose gel at 100V for 1h. After electrophoresis, the DNA was stained with ethidium bromide and visualized under ultraviolet light. A molecular marker of 50 base pairs was used as a size control for the amplified fragments[9].

This first report of *R. stali* infection by *T. rangeli* increases the total number of triatomine vector species of this protozoan from 13 to 14, with 8 of these occurring in Brazil. It is known that the protozoan *T. rangeli* can be found infecting any species of triatomine; however, the only vectors of this trypanosomatid confirmed to date are the species of the genus *Rhodnius*[8]. The report of a 14th species of *Rhodnius* infected by *T. rangeli* is important, since it is known that this protozoan has relevance for the study of Chagas disease, since more than 60% of its antigens are associated with *T. cruzi*[1].

The occurrence of *T. rangeli* naturally infecting *R. stali* offers increased knowledge of the geographical distribution of this parasite in the northern region of Brazil, because it acts as an alert regarding epidemiological surveillance of the same area. Moreover, it is known that the occurrence of *T. cruzi* and *T. rangeli* in the same geographical region, allows for the occurrence of mixed infections in both vertebrate hosts and vectors[1], making it difficult to isolate and differentially diagnose the infection, leading to misdiagnoses of Chagas disease[15]. This situation is of concern, especially in regions where other vector species of *T. rangeli* are reported, such as the state of Acre, where they occur in addition to *R. stali*, for example, the species *R. pictipes, R. robustus* and *R. montenegrensis*[10,14].

Ethical considerations

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.

Conflict of interest

The authors declare that there is no conflict of interest.

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