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ARTÍCULO ORIGINAL

Susceptibility of different *Rhodnius* species (Hemiptera, Reduviidae, Triatominae) to a Brazilian strain of *Trypanosoma rangeli* (SC58/KP1-)

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Introduction: Specific host-parasite associations have been detected experimentally and suggest that triatomines of the genus *Rhodnius* act as biological filters in the transmission of *Trypanosoma rangeli*.
Objective: To analyze the susceptibility of four *Rhodnius* species (*Rhodnius robustus*, *Rhodnius neglectus*, *Rhodnius nasutus* and *Rhodnius pictipes*) to a Brazilian strain of *T. rangeli* (SC-58/KP1-).
Materials and methods: We selected thirty nymphs of each species, which were fed on blood infected with *T. rangeli*. Periodically, samples of feces and hemolymph were analyzed. Triatomines with *T. rangeli* in their hemolymph were fed on mice to check for transmission by bites. Later, the triatomines were dissected to confirm salivary gland infection.
Results: Specimens of *R. pictipes* showed higher rates of intestinal infection compared to the other three species. Epimastigotes and trypomastigotes were detected in hemolymph of four species; however, parasitism was lower in the species of the *R. robustus* lineage. *Rhodnius robustus* and *R. neglectus* specimens did not transmit *T. rangeli* by bite; after dissection, their glands were not infected. Only one specimen of *R. nasutus* and two of *R. pictipes* transmitted the parasite by bite. The rate of salivary gland infection was 16% for *R. pictipes* and 4% for *R. nasutus*.
Conclusions: Both infectivity (intestinal, hemolymphatic and glandular) and transmission of *T. rangeli* (SC58/KP1-) were greater and more efficient in *R. pictipes.* These results reinforce the hypothesis that these triatomines may act as biological filters in the transmission of *T. rangeli*.

Key words: *Trypanosoma rangeli*, *Rhodnius,* host-parasite interactions.

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Sensibilidad de diferentes especies de *Rhodnius* (Hemiptera, Reduviidae, Triatominae) a una cepa brasileña de *Trypanosoma rangeli* (SC58/KP1-)

Introducción. Se han detectado asociaciones biológicas huésped-parásito específicas que sugieren que los triatominos del género *Rhodnius* podrían actuar como filtros biológicos en la transmisión de *Trypanosoma rangeli*.
Objetivo. Estudiar la sensibilidad de cuatro especies de *Rhodnius* (*Rhodnius robustus*, *Rhodnius neglectus*, *Rhodnius nasutus* y *Rhodnius pictipes*) frente a la cepa de *T. rangeli* (SC-58/KP1-).
Materiales y métodos. Se seleccionaron treinta ninfas de cada especie después de xenodiagnóstico artificial en sangre infectada con *T. rangeli.* Se examinaron periódicamente muestras de heces y hemolinfa. Los insectos con hemolinfas infectadas fueron alimentados en ratones a fin de comprobar la transmisión por picadura y posteriormente disecados para confirmar la infección de las glándulas salivales.
Resultados. En *Rhodnius pictipes* se encontró un mayor porcentaje de infección intestinal que en las otras especies. Se detectaron epimastigotes y tripomastigotes en la hemolinfa de las cuatro especies, y se encontró que el parasitismo fue menor en las especies del linaje *R. robustus*. *Rhodnius robustus* y *R. neglectus* no transmitían *T. rangeli* a ratones por picadura: después de la disección, sus glándulas no estaban infectadas. Solo un espécimen de *R. nasutus* y dos de *R. pictipes* transmitieron el parásito por la picadura. La tasa de infección glandular fue de 16 % para *R. pictipes* y de 4 % para *R. nasutus*.

Author's contributions:
Daniella Barreto-Santana: experimental design, data collection in the laboratory, data analysis and the writing of this manuscript
Liliane Santos-Schuenker and Aline Rosa da Fonseca: data collection in the laboratory
Rodrigo Gurgel-Gonçalves and Cesar Augusto Cuba-Cuba: contribution of data bases for each registry, experimental study design, participation in the discussion, drafting and reviewing of this manuscript

Conclusiones. La capacidad infecciosa (hemolinfática, intestinal y glandular) y la transmisión de *T. rangeli* (SC-58/KP1-) fueron mayores y más eficientes en *R. pictipes*. Estos resultados refuerzan la hipótesis de que estos triatominos actúan como filtros biológicos en la transmisión de *T. rangeli*.

Palabras clave: *Trypanosoma rangeli*, *Rhodnius,* interacciones huésped-parásitos.

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Trypanosoma rangeli is a protozoan parasite that has been found in different species of mammals and triatomines throughout South and Central America. Its geographic distribution often overlaps with that of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. Mixed infections with both protozoan species have been detected in different host species and humans, which causes difficulties in the diagnosis of Chagas disease. The most notable biological characteristic of *T. rangeli* in its invertebrate hosts is the passage of parasites from the intestine into the hemolymph and then to the insect salivary glands, where the protozoan develops infective metacyclic trypomastigotes. Then, they become infective to the vertebrate host through the bite of the insect vector (1-3). *Rhodnius* specimens are particularly susceptible to infection with *T. rangeli;* 13 of 19 *Rhodnius* species were reported to be infected with this parasite (4,5).

Biological, biochemical and molecular parameters have demonstrated polymorphism between strains of *T. rangeli* isolated from different triatomines and vertebrate hosts in different geographic regions (6-9). Vallejo, *et al.* (10), characterized two major lineages of *T. rangeli* in Latin America based on the presence or absence of a minicircle of kDNA (KP1+ and KP1-, respectively). *T. rangeli* presents an extensive genetic variability demonstrated by kDNA and nuclear DNA analysis (7,9,11,12). Moreover, analysis of the karyotype profiles permitted the division of the *T. rangeli* strains into two groups coinciding with the KP1+ and KP1− genotypes (13). Subsequent studies clearly showed host-parasite specific associations between *T. rangeli*-*Rhodnius* spp. and a hypothesis that these triatomines act as biological filters in the transmission of genetically distinct populations of *T. rangeli* (11,14,15).

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Abad-Franch and Monteiro (16) and Abad-Franch, *et al.* (17), hypothesized that there are two major evolutionary lineages within the genus *Rhodnius*: the *R. pictipes* lineage, composed by Andean and Amazonian species (such as *R. pallescens*, *R. pictipes, R. colombiensis* and *R. ecuadoriensis*), and the *R. robustus* lineage, composed by Amazonian species (*R. robustus sensu lato* and *R. prolixus*) and species that occur in other ecoregions, such as the caatinga (*R. nasutus*), cerrado (*R. neglectus*), and Atlantic forest (*R. domesticus*). It has been demonstrated that *T. rangeli* strains isolated from species of the *R. pictipes* lineage (KP1-), are genetically divergent to those isolated from the *R. robustus* lineage (KP1+), indicating possible differences in the susceptibility of vectors to different genotypes of *T. rangeli* (11). Such association agrees with the report of a trypanolytic protein present in *R. prolixus* hemolymph, which selectively lyses (KP1-) strains isolated from *R. pallescens*, *R. colombiensis* and *R. ecuadoriensis*, but not (KP1+) strains (18). However, the susceptibility of the species that give the name to these evolutionary lineages (*R. pictipes* and *R. robustus*) has not yet been evaluated.

Arthropod-borne parasites have possibly co-evolved with their biological vectors, especially those that are hematophagous (19). This process is complex and poorly explored in trypanosomatids. Our work offers some insights into this matter. Based on the current hypothesis regarding *T. rangeli* and *Rhodnius* spp. co-evolution (11,14,15), we propose that infectivity (intestinal, hemolymphatic and glandular) and the transmission of the *T. rangeli* strain SC-58 (characterized as KP1-) would be greater and more efficient in *R. pictipes*. Therefore, the goal of the present study was to analyze the susceptibility of four *Rhodnius* species (*R. robustus*, *R. neglectus*, *R. nasutus* and *R. pictipes*) to a Brazilian strain of *T. rangeli* (SC-58/KP1-).

Materials and methods

Insects and T. rangeli strain

Triatomines were obtained from colonies maintained at the Laboratório de Parasitologia Médica e Biologia de Vetores, Faculdade de Medicina, Universidade

de Brasília. Specimens of *R. neglectus* were originally collected in Ituiutaba, in the state of Minas Gerais, *R. nasutus* in Sobral, state of Ceará, whereas the specimens of *R. robustus* were collected in Marabá, state of Pará (20). *Rhodnius pictipes* specimens were obtained from colonies maintained at the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Fiocruz, Rio de Janeiro, and originated from Barcarena, in the state of Pará.

Males and females of each species (*R. robustus*, *R. nasutus*, *R. neglectus* and *R. pictipes*) were housed together in order to obtain eggs (21). After oviposition, 50 eggs of each species were separated. The temperature and relative humidity in the insectary were monitored weekly.

Triatomines were fed by using 30-to-40-day-old Swiss albino mice weighing approximately 30 to 35 g. Mice were obtained from the Bioterium at the Universidade de Brasília. They were anesthetized intraperitoneally with ketamine 80 mg/kg + xylazine 10 mg/kg. Environmental conditions, management and animal care followed standards recommended by the Guide for the Care and Use of Laboratory Animals, and the ethical principles in animal experiments suggested by the Ethics Committee on Animal Use (CEUA), Universidade de Brasília – Instituto de Ciências Biológicas (UnBDoC: 42003/2010).

The Brazilian *T. rangeli* strain SC-58 was used in the study. This strain was isolated by Steindel, *et al.* (22), from a wild rodent, *Echimys dasythrix*, in the state of Santa Catarina. The strain was maintained in culture medium blood agar at 25-28°C and with successive passages made every two weeks. According to Vallejo, *et al.* (23), this strain is characterized molecularly as KP1-.

Experimental infection

Forty specimens of *R. robustus*, *R. pictipes*, *R. nasutus* and *R. neglectus* were fed on blood infected with *T. rangeli*. For that purpose, 3 ml of a SC-58 culture (2.8 x 10^7, mainly epimastigotes) were added to 5 ml of human blood in tubes with heparin, using an artificial feeder (24). At the end of the procedure, 30 nymphs at stages IV and V that engorged with blood were selected, placed individually in a suitable container and labeled.

After seven days of infection, the feces of triatomines were examined microscopically. The stools were obtained by gentle pressure on the last abdominal segments, and the material was diluted in saline solution and freshly examined at 400X magnification. Negative bugs were examined again at 5 to 7 day intervals.

Hemolymphatic samples of triatomines were collected to determine the approximate period of hemocoel invasion by *T. rangeli*. Samples were obtained after a tarsal section of a single leg and they were freshly examined at 400X magnification (25).

Triatomines with positive hemolymph were placed to feed on albino mice 1, 3, 5, 7 and 9 days after first diagnosis to determine the approximate period of salivary gland invasion by *T. rangeli* and transmission by bite (26). Fresh blood samples were obtained from tails of anesthetized mice. Samples were examined at 400X magnification. At the end of the experiment, mice were euthanized in a CO_2 chamber with a concentration above 70%.

Forty-two days after the infective feeding, the salivary glands of the surviving triatomines were extracted and immediately examined to identify the presence of parasites (2). Then, the insects were isolated and subsequently dissected. Finally, their glands were stained with Giemsa after acid hydrolysis (27). The effects of parasitism (rejection of feeding, molting deformations, mortality) were recorded and quantified daily throughout the experiment.

To study the morphogenesis of *T. rangeli* in the intestine, hemolymph and salivary glands, each slide was stained with Giemsa 10% for 45 minutes. During the microscopic observation of the material, the flagellates that most represented the *T. rangeli* life cycle were photographed (Canon: PowerShot SD 700 IS).

Statistical analysis

Chi-square tests were performed to verify differences in the proportion of triatomines infected and not infected by *T. rangeli* among the four *Rhodnius* species studied, considering $p<0.05$ as statistically significant. Proportions and confidence intervals (lower and upper) of infected specimens per species were estimated after 7, 21 and 35 days of experimental infection with *T. rangeli*. The proportions were calculated using the method of Agresti and Coull (28). Separate analyses were conducted for intestinal, glandular and hemolymphatic infection. Moreover, the survival curves of *Rhodnius* species experimental groups were compared using the Kaplan-Meier method. Statistical significance of any differences observed was then assessed by the log rank test. Statistical analysis was performed using SPSS®, v. 20.0 (IBM Corp., Armonk, NY, USA).

Results

Intestinal infection

After 7 days, 15 (50%) specimens of *R. pictipes* had a large number of epimastigotes in the feces (table 1). After 35 days, the proportion of infected specimens of *R. pictipes* differed from the proportions of the other species of *Rhodnius* that were analyzed (p<0.05). *R. pictipes* showed a higher percentage of infected triatomines in a shorter period of time, as well as a gradual increase when compared with the three other species of *Rhodnius* (figure 1).

Hemolymphatic infection

All analyzed *Rhodnius* species showed parasites (epimastigotes and trypomastigotes) in their hemolymph. The low number of insects with hemolymph infection did not allow us to test for differences between the species. Parasitism was less frequent in the species of the *R. robustus* lineage (figure 1). Seven days after infection, *R. nasutus* and *R. neglectus* presented only one specimen each (3.3%) with flagellates in the hemolymph; and after 28 days, they presented a total of four (13.3%) positive specimens. Three specimens of *R. robustus* showed epimastigotes of *T. rangeli* in the hemolymph 42 days after infection (table 1, figure 2).

R. pictipes showed the highest rate of hemolymphatic infection, with four specimens (13.3%) infected after 14 days, totaling seven specimens (23.3%) with infected hemolymph after 28 days (table 1, figure 1, figure 2).

Salivary gland infection and transmission by bite

In total, 25 transmission trials by bite were made with hemolymph-infected specimens. *R. neglectus* and *R. robustus* did not transmit *T. rangeli* after 11 trials. In contrast, two specimens of *R. pictipes*

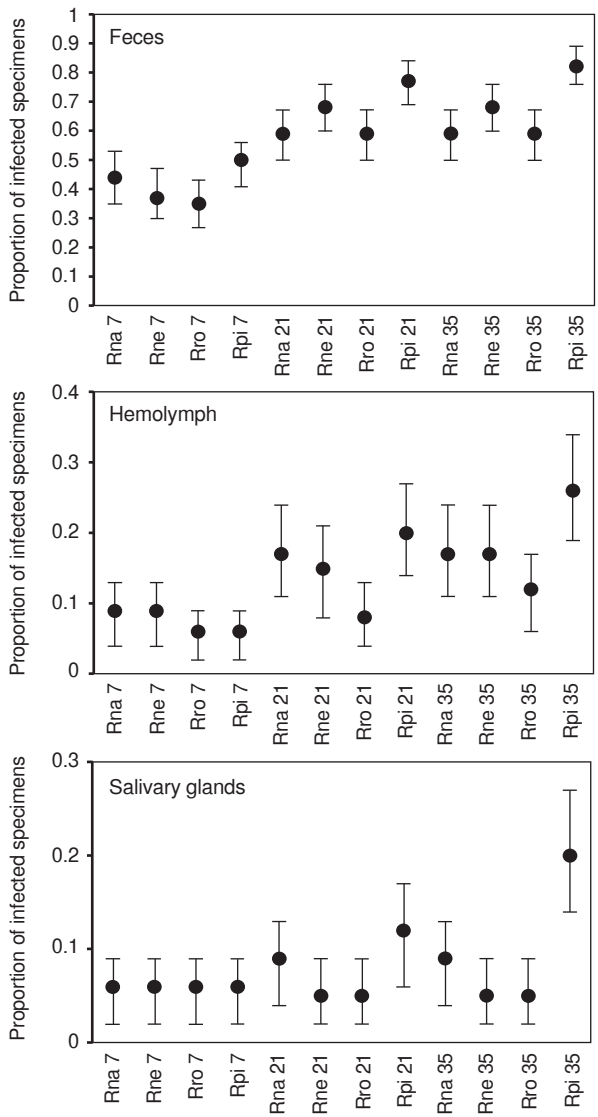

Figure 1. Estimated proportions (points) and confidence intervals (bars) of infected specimens of *Rhodnius* spp. (Rna: *R. nasutus*, Rne: *R. neglectus*, Rro: *R. robustus* and Rpi: *R. pictipes*) after 7, 21 and 35 days of experimental infection with *Trypanosoma rangeli* (SC-58/KP1-)

Table 1. Cumulative number of infected specimens (fecal, hemolymphatic and salivary gland infections) and percentage of mortality of *Rhodnius* spp. after 42 days of experimental infection with *Trypanosoma rangeli* (SC-58/KP1-)

	R. nasutus				*R. neglectus*				*R. robustus*				*R. pictipes*			
Days	**Cumulative number of infected specimens**			**% Mortality**	**Cumulative number of infected specimens**			**% Mortality**	**Cumulative number of infected specimens**			**% Mortality**	**Cumulative number of infected specimens**			**% Mortality**
	Fecal	**Hemo***	**SalGl****		**Fecal**	**Hemo***	**SalGl****		**Fecal**	**Hemo***	**SalGl****		**Fecal**	**Hemo***	**SalGl****	
7	13	1	0	3	11	1	0	7	10	0	0	0	15	0	0	7
14	18	3	0	37	20	2	0	27	16	0	0	0	23	4	1	47
21	18	4	1	57	21	3	0	30	18	1	0	23	24	5	2	73
28	18	4	1	83	21	4	0	63	18	2	0	40	26	7	2	90
35	18	4	1	97	21	4	0	67	18	2	0	63	26	7	5	97
42	18	4	1	97	21	4	0	93	18	3	0	87	26	7	5	97

* Hemolymphatic infection; ** Salivary gland infection

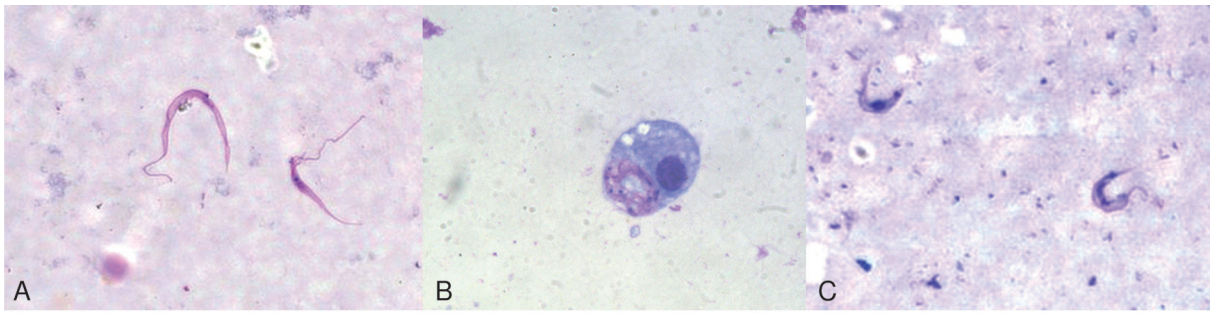

Figure 2. Morphogenesis of *Trypanosoma rangeli* (SC-58/KP1-) in the hemolymph and salivary glands of *Rhodnius* spp. stained with Giemsa (1,000X). A. Epimastigotes within hemolymph samples of *R. robustus*. B. Flagellates within hemocyte of *R. pictipes*. C. Trypomastigotes within salivary gland samples of *R. pictipes*.

Table 2. Results of *Trypanosoma rangeli* (SC58/KP1-) transmission experiment using different *Rhodnius* species

Species	No. of trials	No. of insects with confirmed transmission by bite	Days after hemolymph infection	Days after intestinal infection	No. of insects with infected salivary gland after dissection
R. nasutus	6	1	3	16	1
R. neglectus	8	0	-	-	0
R. robustus	3	0	-	-	0
R. pictipes	8	2	2-3	15-17	3

and one of *R. nasutus* succeeded in transmitting *T. rangeli* via biting after 2-3 days of hemolymphatic infection (table 2, figure 2).

Forty-two days after infection, we extracted the glands of six survivors; only one specimen of *R. nasutus* and two of *R. pictipes* showed glandular infection (table 2).

Mortality rates

High mortality rates were observed during the experiment (table 1). After 28 days, 90% of *R. pictipes* were dead, the highest rate among the four species. A Kaplan-Meier plot of survival is shown in figure 3. There was a significant difference in survival for the *Rhodnius* species analyzed (Log rank = 48.5, $p<0.01$); *R. pictipes* showed the lowest survival rate.

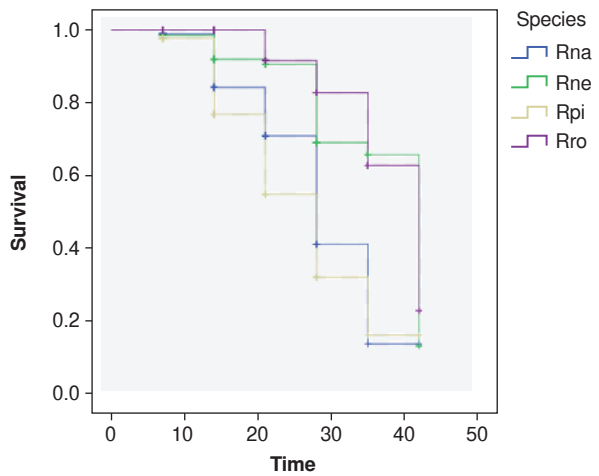

Figure 3. Cumulative survival rate of *R. nasutus* (Rna), *R. neglectus* (Rne), *R. pictipes* (Rpi) and *R. robustus* (Rro) during the time course of infection by *T. rangeli* (SC-58/KP1-) strain

Discussion

The four *Rhodnius* species studied here were susceptible to infection with strain SC-58/KP1- of *T. rangeli* at different levels of outcomes following exposure. Clearly, the genetic profile of both the insect and the parasite must influence the final outcome of host-parasite interactions. *R. pictipes* showed higher rates of intestine, hemolymph and salivary gland infections. These observations play an important role in the transmission of *T. rangeli*. *R. pictipes* appears to offer a compatible biochemical environment for the successful development and reproduction of *T. rangeli* KP1-. Our data on intestinal infection of *R. pictipes* (76.7% in 14 days) were compared with those obtained by Cuba-Cuba (26), who, using a Peruvian strain of *T. rangeli*, obtained 83% of intestinal infections in *R. ecuadoriensis* 15 days after experimental infection.

Machado, *et al.*, (2), using the same strain as in the present paper (SC-58/KP1-), observed glandular infection in *R. nasutus* and *R. neglectus*, but to a lesser extent compared to the other strain (Choachí/

KP1+), which developed much better in these species of the *R. robustus* lineage. We observed the same behavior when comparing the development of strain SC-58. The specimens of the *R. robustus* lineage had a lower percentage of infections in the hemolymph than those of *R. pictipes*. A plausible explanation for this is the immune response of the vector to the parasite. A strong response can reduce or restrict the vectorial capacity of some hosts. The cellular and or humoral components in the hemolymph, for instance, elicited an immune response that can effectively destroy the parasite. In contrast, a weakened response can enhance the ability to support parasite development (29).

The morphogenesis studied followed the pattern expected for *Rhodnius* spp. infections by *T. rangeli* (27,30), with the presence of intestinal epimastigotes, intracellularly dividing flagellates in hemocytes, extracellular epimastigotes and metacyclic trypomastigotes in the salivary glands, in the case of *R. pictipes* infection. The importance of epimastigote forms is well established in the literature suggesting distinct infectivity and metabolic pathways for two parasite morphotypes (long and short) within triatomine bugs. Infections with short epimastigotes of *T. rangeli* are able to mobilize the proteases stored in the fat body, whereas long epimastigotes in some way inhibit protease activities in the fat body (31). Short epimastigotes were more resistant to lysis and stimulated greater superoxide and prophenoloxidase responses than long form epimastigotes of *T. rangeli* (32,33). Moreover, the ecto-phosphatase activity of short forms was more sensitive than that found in the long form (34,35). Finally, long but not short epimastigotes adhered to the gland cells and the strength of interaction correlated with the enzyme activity levels (36). In the present study, we did not characterize the epimastigote forms inoculated in *Rhodnius* specimens. Future studies selecting the forms inoculated during experimental infection should clarify the role of long/short epimastigotes on infectivity for *Rhodnius* specimens.

Isolates of *T. rangeli* from different geographical origins show variable behavior in different *Rhodnius* species; moreover, transmission by biting, as described, is virtually restricted to the local vector (3,37,38). The difference in susceptibility to the strains of *T. rangeli* among the species of *Rhodnius* reinforces the existence of a complex relationship between vector and parasite, and shows that the ability of *T. rangeli* to reach the hemolymph and the salivary glands of the insect is dependent on both the strain used and the triatomine species (2). This study shows for the first time that *R. pictipes* is susceptible to *T. rangeli* strain SC-58/KP1-, which was expected, considering previous hypotheses of parasite-vector coevolution (11,15).

According to Urrea, *et al*. (15), the fact that identical genotypes of *T. rangeli* were isolated in vectors of the same evolutionary lineage supports a possible coevolutionary association between *T. rangeli* and its vectors, which most likely means that these genotypes have the same biological, biochemical and molecular characteristics determining their association with the *Rhodnius* lineages.

Despite the presence of *T. rangeli* in the hemolymph of all four *Rhodnius* species in this study, only *R. pictipes* and *R. nasutus* showed glandular infection. Both species have been reported to be infected naturally. It appears that some triatomine species become infected with *T. rangeli*, which reaches the hemolymph, but never invade the salivary glands. These triatomine species would be permissive to the parasite but they are not able to transmit *T. rangeli* via biting as showed for *Panstrongylus herreri* (39). Evidence of glandular infection of *R. nasutus* by *T. rangeli* (SC-58/KP1-) had already been found by Machado, *et al.* (2), but with a smaller proportion compared to infection with the Choachí strain (KP1+).

With regard to establishing the approximate period in which the SC-58/KP1- strain of *T. rangeli* led to invasion of the salivary glands, our observations are quite similar to those described by Castaño, *et al.* (40), who studied the development of the Choachí strain of *T. rangeli* (KP1+) in *R. prolixus*, observing flagellates in the salivary glands starting only on the 10th day after infection; this infection pattern was also observed by Cuba-Cuba (26) in *R. ecuadoriensis.*

Pulido, *et al.* (18), demonstrated the existence of a trypanolytic protein, which acts against *T. rangeli* (KP1-) isolated from *R. colombiensis*, but not against *T. rangeli* (KP1+) isolated from *R. prolixus*. According to Vallejo, *et al.* (11), the lytic factor in *R. prolixus* hemolymph appears to act as a biological barrier. The occurrence of this protein in other species of the *R. robustus* lineage (*R. robustus, R. neglectus, R. nasutus*) may prevent the glandular development and transmission of *T. rangeli* (KP1-). This suggests that in their natural habitat, some *Rhodnius* species are biological filters for certain populations of parasites, confirming a close coevolutionary association between the two

subpopulations of *T. rangeli* and the two main *Rhodnius* lineages. In addition, the interactions between *T. rangeli* and the triatomine immune system or between *T. rangeli* and symbionts in triatomine intestines may be determinant factors for parasite survival in a particular vector (11).

T. rangeli is considered to be pathogenic to its invertebrate hosts, being responsible for triatomine death (1,6,41,42). It is known that several aspects of the physiology of the triatomines are altered during infection by *T. rangeli*, including reduced immune responses, anti-hemostatic activity of the salivary glands, and behavioral changes such as molting delays, changes in the movements of the insects, decreased reproductive performance and increased mortality (29,43,44). However, the mortality rates in our study were much higher than those observed in other studies analyzing *T. rangeli* experimental infection (2,25). This may be due to the manipulation of the insects during the experiment by their tarsal sections to obtain hemolymphatic samples. Machado, *et al.* (2), also showed that the manipulation of insects in experiments can be harmful to the triatomines, and therefore have an influence on the observed mortality rates.

The infectivity of strain SC-58 of *T. rangeli* (KP1-) was higher in *R. pictipes* compared with the species of the *R. robustus* lineage analyzed (*R. robustus, R. neglectus* and *R. nasutus*), confirming existing hypothesis of coevolution between *T. rangeli* and *Rhodnius* spp. (11,14,15,45). We may assume from these studies that *R. pictipes* is a biological vector of proven competence and vector capacity. Future experimental infections comparing the susceptibility of these species of *Rhodnius* to the population of *T. rangeli* KP1 + may further clarify the role of these triatomines in the transmission of these protozoans in nature.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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References

1. **Cuba-Cuba CA.** Revisión de los aspectos biológicos y diagnósticos del *Trypanosoma (Herpetosoma) rangeli*. Rev Soc Bras Med Trop. 1998;31:207-20. http://dx.doi.org/10.1590/S0037-86821998000200007
2. **Machado PE, Eger-Mangrich I, Rosa G, Koerich LB, Grisard EC, Steindel M.** Differential susceptibility of triatomines of the genus *Rhodnius* to *Trypanosoma rangeli* strains from different geographical origins. Int J Parasitol. 2001;31:632-4. http://dx.doi.org/10.1016/S0020-7519(01)00150-3
3. **Guhl F, Vallejo GA.** *Trypanosoma* (Herpetosoma) *rangeli* Tejera, 1920: An updated review. Mem Inst Oswaldo Cruz. 2003;98:435-42. http://dx.doi.org/10.1590/S0074-02762003000400001
4. **Meneguetti DUO, Soares EB, Campaner M, Camargo LMA.** First report of *Rhodnius montenegrensis* (Hemiptera: Reduviidae: Triatominae) infection by *Trypanosoma rangeli*. Rev Soc Bras Med Trop. 2014;47:374-6. http://dx.doi.org/10.1590/0037-8682-0179-2013
5. **Abad-Franch F, Pavan MG, Jaramillo-O N, Palomeque FS, Dale C, Chaverra D, *et al.*** *Rhodnius barretti*, a new species of Triatominae (Hemiptera: Reduviidae) from western Amazonia. Mem Inst Oswaldo Cruz. 2013;108:92-9. http://dx.doi.org/10.1590/0074-0276130434
6. **D'Alessandro A.** Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920, in: Lumsden, W.H.R., Evans, D.A. (Eds.), Biology of Kinetoplastida, Vol. 1. London: Academic Press; 1976. p. 327-493. http://dx.doi.org/10.1111/j.1550-7408.1977.tb04770.x
7. **Steindel M, Dias-Neto E, Carvalho-Pinto CJ, Grisard E, Menezes C, Murta SMF, *et al.*** Randomly amplified polymorphic DNA (RAPD) and isoenzyme analysis of *Trypanosoma rangeli* strains. J Eukaryot Microbiol. 1994;41:261-7. http://dx.doi.org/10.1111/j.1550-7408.1994.tb01506.x
8. **Grisard EC, Steindel M, Guarneri AA.** Characterization of *Trypanosoma rangeli* strains isolated in Central and South America: An overview. Mem Inst Oswaldo Cruz. 1999;94:203-9. http://dx.doi.org/10.1590/S0074-02761999000200015
9. **Maia da Silva F, Junqueira AC, Campaner M, Rodrigues AC, Crisante G, Ramirez LE, *et al.*** Comparative phylogeography of *Trypanosoma rangeli* and *Rhodnius* (Hemiptera: Reduviidae) supports a long coexistence of parasite lineages and their sympatric vectors. Mol Ecol. 2007;16:3361-73. http://dx.doi.org/10.1111/j.1365-294X.2007.03371.x
10. **Vallejo GA, Guhl F, Carranza JC, Lozano LE, Sánchez JL, Jaramillo JC, *et al.*** kDNA markers define two major *Trypanosoma rangeli* lineages in Latin America. Acta Trop. 2002;81:77-82. http://dx.doi.org/10.1016/S0001-706X(01)00186-3
11. **Vallejo GA, Guhl F, Schaub GA.** Triatominae-*Trypanosoma cruzi*/*T. rangeli*: Vector-parasite interactions. Acta Trop. 2009;110:137-47. http://dx.doi.org/10.1016/j.actatropica.2008.10.001

12. **Stoco PH, Wagner G, Talavera-López C, Gerber A, Zaha A, Thompson CE, *et al.*** Genome of the Avirulent Human-Infective Trypanosome-*Trypanosoma rangeli.* PLoS Negl Trop Dis. 2014;8:e3176. http://dx.doi.org/10.1371/journal.pntd.0003176

13. **Cabrine-Santos M, Ferreira KA, Tosi LR, Lages-Silva E, Ramirez LE, Pedrosa AL.** Karyotype variability in KP1(+) and KP1(-) strains of *Trypanosoma rangeli* isolated in Brazil and Colombia. Acta Trop. 2009;110:57-64. http://dx.doi.org/10.1016/j.actatropica.2009.01.004

14. **Urrea DA, Carranza JC, Cuba-Cuba CA, Gurgel-Gonçalves R, Guhl F, Schofield CJ, *et al.*** Molecular characterization of *Trypanosoma rangeli* strains isolated from *Rhodnius ecuadoriensis* in Perú, *R. colombiensis* in Colombia and *R. pallescens* in Panamá supports a co-evolutionary association between parasites and vectors. Infect Genet Evol. 2005;5:123-9. http://dx.doi.org/10.1016/j.meegid.2004.07.005

15. **Urrea DA, Guhl F, Herrera CP, Falla A, Carranza JC, Cuba-Cuba CA, *et al.*** Sequence analysis of the spliced-leader intergenic region (SL-IR) and random amplified polymorphic DNA (RAPD) of *Trypanosoma rangeli* strains isolated from *Rhodnius ecuadoriensis*, *R. colombiensis*, *R. pallescens* and *R. prolixus* suggests a degree of co-evolution between parasites and vectors. Acta Trop. 2011;120:59-66. http://dx.doi.org/10.1016/j.actatropica.2011.05.016

16. **Abad-Franch F, Monteiro FA.** Biogeography and evolution of Amazonian triatomines (Heteroptera: Reduviidae): Implications for Chagas disease surveillance in humid forest ecoregions. Mem Inst Oswaldo Cruz. 2007;102:57-70. http://dx.doi.org/10.1590/S0074-02762007005000108

17. **Abad-Franch F, Monteiro FA, Jaramillo ON, Gurgel-Gonçalves R, Dias FB, Diotaiuti L.** Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: A multi-scale appraisal of the tribe *Rhodniini* (Triatominae). Acta Trop. 2009;110:159-77. http://dx.doi.org/10.1016/j.actatropica.2008.06.005

18. **Pulido XC, Pérez G, Vallejo GA.** Preliminary characterization of a *Rhodnius prolixus* hemolymph trypanolytic protein, this being a determinant of *Trypanosoma rangeli* KP1(+) and KP1(-) subpopulations' vectorial ability. Mem Inst Oswaldo Cruz. 2008;103:172-9. http://dx.doi.org/S0074-02762008000200008

19. **Clayton DH, Johnson KP.** Linking coevolutionary history to ecological process: Doves and lice. Evolution. 2003;57:2335-41. http://dx.doi.org/10.1554/03-123

20. **Gurgel-Gonçalves R, Abad-Franch F, Ferreira JBC, Barreto-Santana D, Cuba-Cuba CA.** Is *Rhodnius prolixus* (Triatominae) invading houses in central Brazil? Acta Trop. 2008;107:90-8. http://dx.doi.org/10.1016/j.actatropica.2008.04.020

21. **Rocha DS, Jurberg J, Galvão C.** Biologia do *Rhodnius pictipes* Stal, 1872 em condições de laboratório (Hemiptera, Reduviidae, Triatominae). Mem Inst Oswaldo Cruz. 1994;89:265-70. http://dx.doi.org/10.1590/S0074-02761994000200028

22. **Steindel M, Carvalho-Pinto CJ, Toma HK, Mangia RHR, Ribeiro-Rodrigues R, Romanha AJ.** *Trypanosoma rangeli* Tejera, 1920 isolated from a sylvatic rodent (*Echimys dasythrix*) in Santa Catarina State: First report of this trypanosome in southern Brazil. Mem Inst Oswaldo Cruz. 1991;86:73-9. http://dx.doi.org/10.1590/S0074-02761991000100012

23. **Vallejo GA, Guhl F, Carranza JC, Triana O, Pérez G, Ortiz PA, *et al.*** Interacción tripanosoma-vector-vertebrado y su relación con la sistemática y la epidemiología de la tripanosomiasis americana. Biomédica. 2007;27:110-8. http://dx.doi.org/10.7705/biomedica.v27i1.254

24. **Cuba-Cuba CA, Alvarenga NJ, Barretto AC, Marsden PD, Chiarini C.** New comparative studies between *Dipetalogaster maximus* and *Triatoma infestans* in the xenodiagnosis of chronic human Chagas infection. Rev Inst Med Trop. 1978;20:145-51.

25. **Marquez DS, Rodrigues-Ottaiano C, Oliveira RM, Pedrosa AL, Cabrine-Santos M, Lages-Silva E, *et al.*** Susceptibility of different triatomine species to *Trypanosoma rangeli* experimental infection. Vector Borne Zoonotic Dis. 2006;6:50-6. http://dx.doi.org/10.1089/vbz.2006.6.50

26. **Cuba-Cuba CA.** Estudo de uma cepa peruana de *Trypanosoma rangeli* II. Observações sobre a infecção experimental de *Rhodnius ecuadoriensis.* Rev Inst Med Trop. 1974;16:19-27.

27. **Cuba-Cuba CA.** Estudo de uma cepa peruana de *Trypanosoma rangeli* IV. Observações sobre a evolução e morfogênese do *T. rangeli* na hemocele e nas glândulas salivares de *Rhodnius ecuadoriensis.* Rev Inst Med Trop. 1975;17:284-97.

28. **Agresti A, Coull BA.** Approximate is better than "exact" for interval estimation of binomial proportions. Amer Statist. 1998;52:119-26. http://dx.doi.org/10.1080/00031305.1998.10480550

29. **Garcia ES, Castro DP, Figueiredo MB, Azambuja P.** Parasite-mediated interactions within the insect vector: *Trypanosoma rangeli* strategies. Parasit Vectors. 2012;5:105. http://dx.doi.org/10.1186/1756-3305-5-105

30. **Paim RM, Pereira MH, Araújo RN, Gontijo NF, Guarneri AA.** The interaction between *Trypanosoma rangeli* and the nitrophorins in the salivary glands of the triatomine *Rhodnius prolixus* (Hemiptera; Reduviidae). Insect Biochem Mol Biol. 2013;43:229-36. http://dx.doi.org/10.1016/j.ibmb.2012.12.011

31. **Feder D, Gomes S, Garcia E, Azambuja P.** Metalloproteases in *Trypanosoma rangeli*-infected *Rhodnius prolixus*. Mem Inst Oswaldo Cruz. 1999;94:771-7. http://dx.doi.org/10.1590/S0074-02761999000600011

32. **Whitten MM, Mello CB, Gomes SA, Nigam Y, Azambuja P, Garcia ES, *et al.*** Role of superoxide and reactive nitrogen intermediates in *Rhodnius prolixus* (Reduviidae)/*Trypanosoma rangeli* interactions. Exp Parasitol. 2001;98:44-57. http://dx.doi.org/10.1006/expr.2001.4615

33. **de Sousa MA, Dos Santos-Pereira SM, Dos Santos-Faissal BN.** Variable sensitivity to complement-mediated lysis among *Trypanosoma rangeli* reference strains. Parasitol Res. 2012;110:599-608. http://dx.doi.org/10.1007/s00436-011-2528-8

34. **Cosentino-Gomes D, Russo-Abrahão T, Fonseca-de-Souza AL, Ferreira CR, Galina A, Meyer-Fernandes JR.** Modulation of *Trypanosoma rangeli* ecto-phosphatase activity by hydrogen peroxide. Free Radic Biol Med. 2009;47:152-8. http://dx.doi.org/10.1016/j.freeradbiomed. 2009.04.020

35. **Gomes SAO, Fonseca-de-Souza AL, Silva BA, Kiffer-Moreira T, Santos-Mallet JR, Santos ALS, *et al.*** *Trypanosoma rangeli*: Differential expression of cell surface

polypeptides and ecto-phosphatase activity in short and long epimastigote forms. Exp Parasitol. 2006;112:253-62. http://dx.doi.org/10.1016/j.exppara.2005.11.015

36. **Dos-Santos AL, Dick CF, Alves-Bezerra M, Silveira TS, Paes LS, Gondim KC, *et al.*** Interaction between *Trypanosoma rangeli* and the *Rhodnius prolixus* salivary gland depends on the phosphotyrosine ecto-phosphatase activity of the parasite. Int J Parasitol. 2012;42:819-27. http://dx.doi.org/10.1016/j.ijpara.2012.05.011

37. **D'Alessandro A, Saravia NG.** *Trypanosoma rangeli*, in: Gilles, H.M. (Ed.), Protozoal Diseases. New York: Oxford University Press; 1999. p. 398-412.

38. **Vallejo GA, Guhl F, Carranza JC, Moreno J, Triana O, Grisard EC.** Parity between kinetoplast DNA and miniexon gene sequences supports either clonal evolution or speciation in *Trypanosoma rangeli* strains isolated from *Rhodnius colombiensis*, *R. pallescens* and *R. prolixus* in Colombia. Infect Genet Evol. 2003;3:39-45. http://dx.doi.org/10.1016/S1567-1348(02)00150-8

39. **Cuba-Cuba CA.** Evolução de uma cepa peruana de *Trypanosoma rangeli* em *Rhodnius ecuadoriensis* e *Panstrongylus herreri*. [Dissertação de Mestrado]. Belo Horizonte: Universidade Federal de Minas Gerais; 1973.

40. **Castaño MTP, Moreno MM, Martí CAZ.** Estudio del comportamiento de dos cepas de *Trypanosoma rangeli*. MedUNAB. 2001;4:166-72.

41. **Grewal MS.** Pathogenicity of *Trypanosoma rangeli*, Tejera 1920, in the invertebrate host. Exp Parasitol. 1957;6:123-30. http://dx.doi.org/10.1016/0014-4894(57)90010-3

42. **Watkins R.** Histology of *Rhodnius prolixus* infected with *Trypanosoma rangeli.* J Invertebr Pathol. 1971;17:59-66. http://dx.doi.org/10.1016/0022-2011(71)90126-1

43. **Ferreira LL, Lorenzo MG, Elliot SL, Guarneri AA.** A standardizable protocol for infection of *Rhodnius prolixus* with *Trypanosoma rangeli*, which mimics natural infections and reveals physiological effects of infection upon the insect. J Invertebr Pathol. 2010;105:91-7. http://dx.doi.org/10.1016/j.jip.2010.05.013

44. **Fellet MR, Lorenzo MG, Elliot SL, Carrasco D, Guarneri AL.** Effects of Infection by *Trypanosoma cruzi* and *Trypanosoma rangeli* on the Reproductive Performance of the Vector *Rhodnius prolixus*. PLoS ONE. 2014;9:e105255. http://dx.doi.org/10.1371/journal.pone.0105255

45. **Salazar-Antón F, Urrea DA, Guhl F, Arévalo C, Azofeifa G, Urbina A, *et al.*** *Trypanosoma rangeli* genotypes association with *Rhodnius prolixus* and *R. pallescens* allopatric distribution in Central America. Infect Genet Evol. 2009;9:1306-10. http://dx.doi.org/10.1016/j.meegid.2009.09.002