Short Report: *Trypanosoma cruzi* Infection in *Mepraia gajardoi* and *Mepraia spinolai*: The Effect of Feeding Nymphs from the Field

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Abstract. We evaluated *Trypanosoma cruzi* infection rates by means of minicircle DNA-based polymerase chain reactions (PCRs) in 70 starved *Mepraia gajardoi* from northern Chile and 65 *M. spinolai* from central Chile after feeding. Immediately after collection in the field, 20% of *M. gajardoi* were found infected; after feeding, 67% of the uninfected were infected. One group of *M. spinolai* seemed to be completely uninfected, but after the first and second feedings, 62% and 59% were positive, respectively.

The protozoan *Trypanosoma cruzi* (Kinetoplastida Trypanosomatidae), etiologic agent of Chagas disease, is mainly transmitted to mammals by contamination of the skin lesions with triatomine insect feces with parasites.¹ In a regularly fed infected triatomine, *T. cruzi* amplify as epimastigotes, which later, differentiate to trypomastigotes that are eliminated by the feces. Several biological factors modulate *T. cruzi*–insect vector interactions, including food supply, intestinal components, gut flora, *T. cruzi* strain, and insect physiology.² The Triatominae subfamily is composed of 146 species distributed in 18 genera and grouped into six tribes.^{3,4} Important vectors rapidly defecate on the host. These vectors urinate/defecate during or after feeding from a rapid to a delayed behavior according the triatomine species.⁵ Several factors, such as the competition of enterobacteria of the flora and *T. cruzi* with its vector for nutrients, and thereby, feeding affect not only parasite density and insect molting but also, changes in the epimastigote/trypomastigote ratio in the rectum of *Triatoma infestans* (Hemiptera, Triatominae).⁶ The rectal parasite density increases until several weeks post-infection with regular blood meals, reaching maximal values of several million parasites. The parasite density is only strongly influenced by very long starvation up to 20 weeks.^{7–9} In the laboratory, the feeding status also modulated the olfactory host search behavior of triatomines.¹⁰ In Chile, the wild vector of Chagas disease is *Mepraia* spp. (Hemiptera, Triatominae), which is composed of three different species: *M. gajardoi*, *M. spinolai*, and *M. parapatrica*.^{11–13} *M. gajardoi* and *M. spinolai* are frequently found in corrals of domestic animals and stony hills and rock crevices of arid zones of the northern and semiarid areas of central Chile, respectively. Between 11% and 27% infections with *T. cruzi* were found in *M. gajardoi*.¹⁴ Infection rates in *M. spinolai* range from 46% to 54%.¹⁵ In the national surveillance program of triatomine vectors, 11–47% of these species were infected.¹⁶ Thus, the aim of this study is to determine *T. cruzi* infection in *M. gajardoi* and *M. spinolai* by assessing infection on insects under natural conditions (right after collection) and reassessing after feeding in the laboratory.

In the laboratory overall, 70 *M. gajardoi* nymphs stages III–V from Vitor and 35 and 30 *M. spinolai* nymphs stages

III–V from Illapel and Colina, respectively, were studied. The insects were maintained and fed individually inside a climate chamber at 27°C with a relative humidity of 70% and a 14:10-hour light:dark photoperiod. Triatomines fed on *Mus musculus* 1 or 2 days after arrival, and the first-drop fecal/urine sample was obtained from each individual. This first feeding allowed us to obtain a sample to determine the infective status right after collection. A second sample was obtained after molting and feeding 4–5 weeks later to assess changes in infective status, and finally, a third sample was obtained 4–5 weeks later from those insects that had survived and fed. *T. cruzi* contained kinetoplast DNA (kDNA) composed of several maxicircle copies and minicircles DNA. Minicircles, because of their abundance, are a perfect molecular marker for parasite detection. *T. cruzi* infection was evaluated by detection of minicircle DNA by polymerase chain reaction (PCR).^{1,17} Therefore, the product of 330 base pairs (bp) indicated a positive result. Each specimen sample was assayed two times. Samples with one positive result and one negative result were considered discordant. Samples with two positive or negative results were considered positive and negative, respectively.

After the first feeding in the laboratory, the PCR analyses with the first fecal samples of *M. gajardoi* from Vitor showed an infection in 14 of 70 samples (Table 1). After the second feeding, 33 of the initially negative nymphs were proven to be infected (Table 1), and another 7 nymphs gave PCR discordant results; up to this feeding, seven nymphs had died, and nine nymphs were negative (nymphs remaining uninfected). As a whole, 47 nymphs were positive. The 14 *M. gajardoi* specimens detected as positive in the first determination maintained that infective status. Overall, in Vitor, 67% of the analyzed samples previously detected as negative were positive after feeding. These differences were significant (χ^2 $P < 0.001$). Investigating 35 *M. spinolai* from Illapel, all first samples contained no trypanosomes, but after the second feeding, 22 of 32 surviving nymphs were positive, 9 nymphs were discordant, and 1 nymph remained negative. Overall, in Illapel, 62% of the analyzed samples were positive after two feedings. This group of insects was studied after a new feeding event, and the results were 19 positive, 6 discordant, and 4 negative samples (3 negatives nymphs died before the third feeding). In this longitudinal study of the analyzed insect samples, 59% were positives (Table 1). All of the *M. spinolai* individuals from Colina remained negative after two successive feedings

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TABLE 1
Number of *T. cruzi*-infected and uninfected nymphs in Triatomines after being fed three times

Triatomine species	Collection locality (number of insects)	First feeding			Second feeding				Third feeding			
		P	D	N	P	D	N	Died	P	D	N	Died
<i>M. gajardoi</i>	Vitor (70)	14	0	56	47	7	9	7	ND	ND	ND	ND
<i>M. spinolai</i>	Illapel (35)	0	0	35	22	9	1	3	19	6	4	3
<i>M. spinolai</i>	Colina (30)	0	0	30	0	0	30	0	ND	ND	ND	ND

Mitochondrial DNA amplification of *T. cruzi* by PCR. D = discordant result in the replica of PCR assays; N = negative; ND = not determined; P = positive.

on mice. Previous results on unfed *M. gajardoi* collected in Vitor reported 15% *T. cruzi*-infected insects overall.¹⁴ In this study, we detected 20% of insects infected right after collection and 67% or even higher of insects infected after feeding if some discordant cases are considered infected with low *T. cruzi* burden. In Illapel, a first study found infection rates of 46–54%,¹⁵ and a second survey in neighbored areas but two ecologically different sites gave rates of 40% and 76%.¹⁸ The infection rate was higher in an area of higher vegetation cover, suggesting that *M. spinolai* in this ecotop has better opportunities to feed on wild animals and therefore, higher infection rates. These investigations in this geographical area initially detected no *T. cruzi* infection in insects, but after the first and second feedings, 62% and 59% or more, respectively, of the nymphs were infected if some discordant cases are considered as infected triatomines. Finally, results from the last endemic area, Colina locality, were surprising. Previous results of infection rates in *M. spinolai* from this locality showed spatial heterogeneity¹⁹ when infection was assessed simultaneously in four different sites. Unlike our study, in that study, *T. cruzi* infection was evaluated by microscopic examination, and infection rates varied up to 34% according the study site. However, this endemic area recently changed from a rural to an urban condition; presumably, human disturbance, including local extinction of competent mammal hosts, could explain this large difference in the level of infection. *M. spinolai* has a patchy distribution. This information was strengthened by a study of the home range of *M. spinolai* that showed very low dispersal capacity, although it was higher in hot seasons.²⁰

More recently, the phylogeny of *Mepraia* spp. was studied all over the country, and a highly structured phylogeography indicated an absence of gene flow between very close insect colonies.²¹ The major reason might be a peculiarity of *Mepraia*, in which females are wingless. As a whole, most of the insect collections in all of these studies were performed with a passive method using humans as baits, which mainly allows the collection of unfed specimens. This observation may be epidemiologically relevant, because these unfed vectors have low *T. cruzi* burden, which has been evident here. In established experimental infections, *T. infestans* remained to be infected after prolonged starvation.⁷ The wild insect vectors in Chile can be highly infected with *T. cruzi*, but those looking for humans to feed are starved with low parasite burden and therefore, have a lower chance of transmitting *T. cruzi*. However, the proximity of other reservoirs (vertebrates) to human housing may change this probability. The second feature that makes *M. spinolai* a vector of low transmissibility is the delayed defecation behavior during feeding compared with other competent vectors, such as *T. infestans*.⁵ However, in infected insects, the period of time before defecation is shortened to a few minutes.²² Although *Mepraia* spp.

seems to be a poor vector of Chagas disease, the presence of these insects in areas of close proximity to human dwellings cannot be underestimated and represents a threat for human populations. Therefore, it is important to examine fluctuations of parasite populations at different stages of their development in different vector species that undergo starvation and famines for longer periods for the purpose of generating knowledge about the maintenance of parasites and future transmission to humans.

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