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First Report of Widespread Wild Populations of *Triatoma infestans* (Reduviidae, Triatominae) in the Valleys of La Paz, Bolivia

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Abstract. Wild populations of *Triatoma infestans*, the main vector of Chagas disease in the Southern Cone countries, may be involved in reinfestation of human dwellings, limiting the success of vector-control campaigns in Bolivia. Knowledge of the distribution of these populations remains incomplete. We report here the detection of *T. infestans* wild populations in large areas in the department of La Paz, Bolivia. Among 18 sylvatic areas investigated, 17 were positive with *T. infestans* specimens. The infection rate of captured *T. infestans* with *Trypanosoma cruzi* was 85.7% in adult specimens. These results expand the geographical distribution of wild populations of *T. infestans*; it may be distributed throughout the Inter-Andean Dry Forest eco-region of Bolivia. The current information allows us to propose the hypothesis that a sylvatic origin of the reinfestation is located in the valleys of La Paz.

INTRODUCTION

Chagas disease is a major public health problem in Latin America. *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), the causative agent of the disease, is transmitted by contaminated feces of triatomine bugs. In Bolivia and other countries of the Southern Cone in South America, *Triatoma infestans* (Klug 1834) remains the main vector species.

In the Southern Cone, control of Chagas disease is being pursued by elimination of *T. infestans* (principally through insecticidal spraying). Control efforts have relied on the assumption that this species was almost exclusively domestic, although a few wild populations had been reported in the Cochabamba valley in Bolivia.[1–3] More recently, other wild foci of *T. infestans* have been described, however, indicating that wild populations of *T. infestans* have a larger dispersion than previously assumed.[4–6] As new species or populations tend to invade the ecological niche released by a first species, wild populations of *T. infestans* could infest habitats free of domestic triatomine populations and jeopardize vector-control efforts.[7,8]

We found wild populations of *T. infestans* in two valleys of La Paz, Bolivia, where transmission of *Trypanosoma cruzi* persists. New hypotheses about the geographical distribution of wild populations of *T. infestans* and the epidemiological risk that they represent are suggested.

MATERIALS AND METHODS

Study areas. In conjunction with the National Program of Chagas disease (PNCH), two valleys under vector control since 2003, Rio La Paz and Rio Luribay, were chosen. Despite insecticidal spraying, locals were still suffering domiciliary reinfestation by *T. infestans*. In these valleys, 6 and 12 trapping areas between 2,159 and 2,864 m altitude were selected (Figure 1; Table 1). All areas were situated in wild environments, which will be referred as "sylvatic" in this work, at a distance varying from 20 to 500 m from human dwellings. Fields closest to rivers are dedicated to vegetable crops and maize in the two valleys. Surrounding mountains have scarce vegetation dominated by thorny plants and cacti. People also cultivate prickly pear (*Opuntia ficus-indica*) and the cochineal (*Dactylopius coccus*) insect used for food coloring and cosmetics. Both valleys are situated in the Inter-Andean Dry Forest eco-region.[9] Climate in this area is semi-arid with an average annual temperature of 18°C (range = 8.3–30.8°C), and mean relative humidity is 5.8%. Rainfall occurs between December and April, and winter and spring are very dry (June to November).

Survey of human population. During April and May 2008, 305 inhabitants from 31 different villages in these valleys were questioned about their knowledge of triatomine bugs (locally called *vinchucas*) in domestic/sylvatic environments and their localization. They were also asked if they have ever seen *vinchucas* flying.

Collection of wild triatomines and laboratory processing. Collection was carried out between May and October 2008 using mice-baited adhesive traps.[10] The traps were placed in small caves and cracks, in burrows, or under vegetation. They were set in the afternoon and inspected the next morning. More than 20 traps were placed in most of the trapping areas (Table 1). All insects caught by a trap were placed together in one tube, and they were transported alive to the laboratory. The identification of triatomines (adults and nymphs) was performed according to morphological taxonomic keys.[11] Sex and stages were also determined. Feces from each bug were examined for the presence of trypanosomatides by direct microscopical observation at 400× magnification. Bugs were then dissected under a safety hood, and legs and digestive tracks were conserved for further studies.

RESULTS

Knowledge of the triatomine bugs by the surveyed population. More than 96% of interviewed locals recognized the vector known as *vinchuca* in the two valleys (Rio La Paz = 117 interviews; Rio Luribay = 188 interviews). Triatomines have been observed in 12% of interviewees' homes within the previous 12 months, and 7% have seen them during the prior month. Moreover, 9% have seen bugs during the year in their peridomicile. In addition, 39.1% of women and 63% of men

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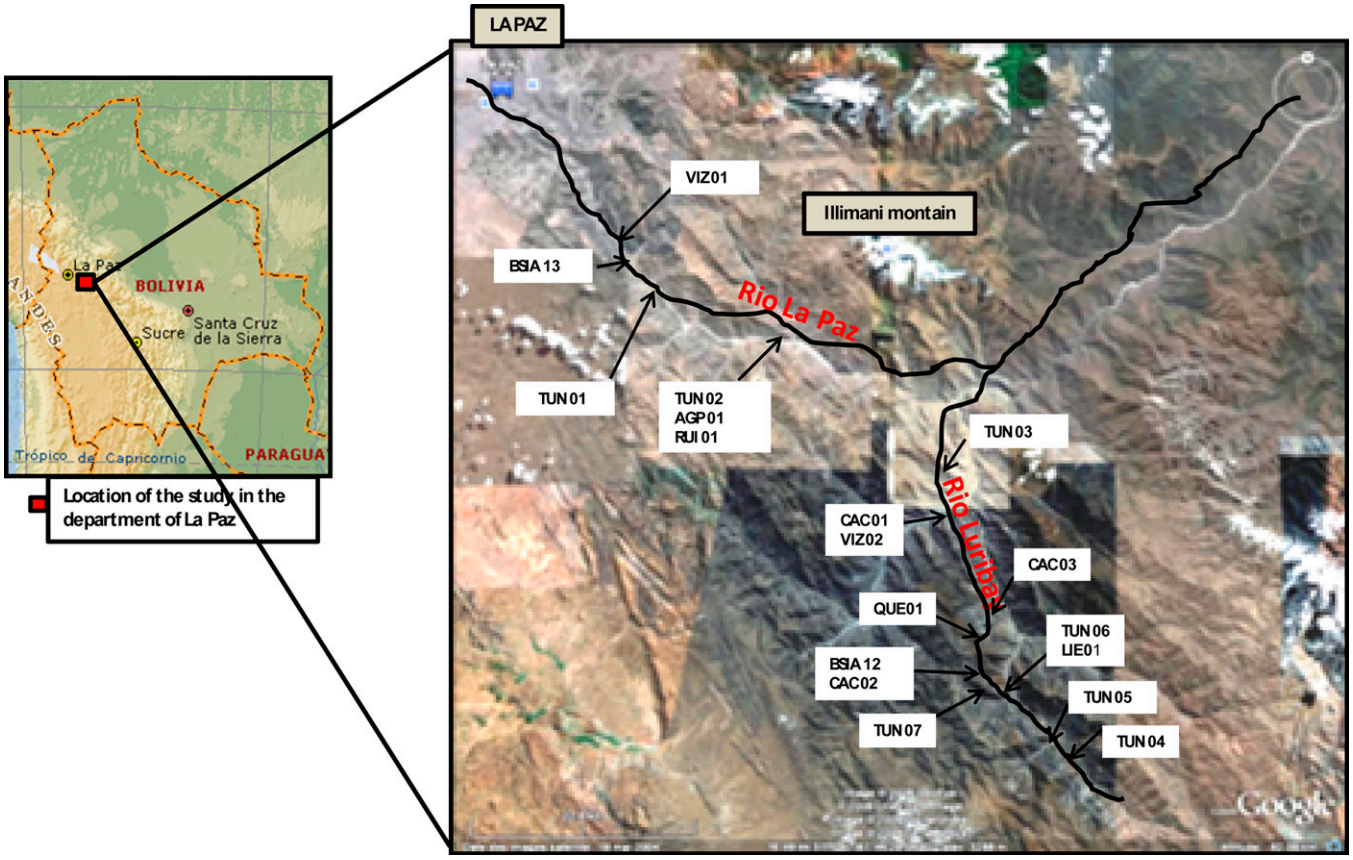

FIGURE 1. Map of Rio La Paz and Rio Luribay valleys (lines) obtained from Google Earth. All the studied areas are located between VIZ 01 (2,821 m) and RUI 01 (2,459 m) and between TUN 04 (2,767 m) and TUN 03 (2,095 m) in Rio La Paz and Rio Luribay, respectively. This figure appears in color at www.ajtmh.org.

surveyed claimed to have seen bugs in the sylvatic environment: in the hills, under rocks, in fields of prickly pear, in ruins, and in cemeteries. Finally, 30% of those surveyed reported that they have seen the bugs flying. "They fly from the hill towards the houses, they fly to the roof of the houses or to the window in the night, and we hear them arriving to the tinny roof" were expressions frequently used by the interviewees.

Areas of collection. Triatomine bugs were found in all trapping areas except one (area CAC 01) in which only nine traps have been placed (Table 1). A total of 646 traps were used during the study, and 29.6% of them caught wild *T. infestans* specimens. The proportion of positive traps in an area was variable and ranged from 3.3% in TUN 07 to 83.7% in area CAC 03, but only two areas had fewer than 5% positive. The five areas with greatest infestation (> 37.5% of traps were positive; Figure 2A–E) were wilderness areas with thorny vegetation and prickly pear fields with rock-pile boundary walls (areas TUN 02, CAC 03, and LIE 01), a ruin with mud walls providing shelter for small rodents (area RUN 01), and a mountainside composed of large rocks where excrements of *vizcachas* (rodents belonging to the Chinchillidae family) were observed (area VIZ 02).

Interestingly, in the positive area VIZ 01 (small highland), traps were placed in deep cracks formed in sediment by erosion (2–5 m) that serve as shelters for *vizcachas* (Figure 2F).

Triatomine bug collection and infection rate. A total of 978 *T. infestans* specimens were collected in 17 positive areas. The number of triatomines by positive trap ranged from 1.4 triatomines per trap in areas QUE 01 and CAC 02 to 9.16 triatomines per trap in area VIZ 02. Adult insects as well as nymphal instars were caught in all areas, and a predominance of young nymphs was observed among the total population (1st + 2nd + 3rd nymphal instars = 70.1%; Figure 3). The proportion of positive traps was significantly higher in Rio Luribay than in Rio La Paz (χ^2 $P < 0.001$), but triatomine density per positive trap was not significantly different (Mann–Whitney–Wilcoxon test, $P > 0.05$). A total of 448 *T. infestans* feces were examined, and positive samples for trypanosomatide were found in all the trapping areas (Table 2). The infection rates ranged from 3.8% in first nymphal instar to 85.7% in adult insects with no significant differences between sex (Figure 4; χ^2 $P > 0.05$). Preliminary analysis of feces by polymerase chain reaction (PCR) confirmed infection of *T. infestans* by *T. cruzi* species (data not shown).[12]

DISCUSSION

Since the first identification of a wild population of *T. infestans* in 1946, several reports have described the existence of other wild foci in the department of Cochabamba, Bolivia.[1–5] Subsequent reports have suggested a wider distribution of wild *T. infestans* in Bolivia than just the Cochabamba valley, and findings of wild *T. infestans* in Argentina (LA Ceballos and others, unpublished data) and Chile have also been reported.[8,13]

TABLE 1

Description, geographical localization, and infestation of the investigated sylvatic areas in two valleys of the department of La Paz, Bolivia

Valley	Location code	Nearest village	Description of trapping areas (distance from nearest habitat)	Latitude	Longitude	Alt. (m)	Date of trapping	Number of traps placed	Number of positive traps	% of positive traps	Total number of triatomine bugs
Rio La Paz	VIZ 01	Aucani	Deep cracks in a small highland that are shelters for vizcachas (Andean small rodent) (200 m).	16°41'25.47"	68°00'39.76"	2,821	5/8/2008	13	2	15.4	4
							8/20/2008	25	6	24.0	22
	BSIA 13	El Palomar	Wilderness mountainside with sparse vegetation of mainly cacti and thorny vegetation (50–200 m).	16°42'02.75"	67°59'54.91"	2,765	9/10/2008	48	4	8.3	22
	TUN 01	Huayhuasi	Field of prickly pears adjacent to some houses outside the village (20–150 m).	16°42'25.47"	67°59'37.64"	2,757	5/8/2008	30	6	20.0	46
							7/16/2008	49	9	18.4	59
							8/19/2008	30	8	26.7	50
	AGP 01	Tahuapalca	Edges of footpaths between crop fields around the village (20–200 m).	16°43'05.03"	67°52'30.59"	2,380	5/10/2008	23	1	4.3	2
	TUN 02	Tahuapalca	Field of prickly pears overhanging the village (50–150 m).	16°43'11.53"	67°52'25.70"	2,427	5/11/2008	29	12	41.4	37
	RUI 01	Tahuapalca	Ruin of a 19th century barracks with mud walls next to a small cemetery on a highland overhanging the village of Tahuapalca (500 m).	16°42'56.45"	67°52'13.53"	2,459	5/10/2008	40	15	37.5	34
	Total Rio La Paz							287	63	22.0	276
Rio Luribay	TUN 04	Carayapu	Wilderness mountainside with sparse vegetation of mainly cacti and thorny vegetation, which is adjacent to some house of the village (50–300 m).	17°08'10.80"	67°35'17.90"	2,767	6/17/2008	30	6	20.0	10
	TUN 05	Pucuma	Wilderness mountainside and cemetery outside the village (100–300 m).	17°07'32.00"	67°35'59.50"	2,864	6/17/2008	20	6	30.0	14
	LIE 01	Cuty	Rock-pile boundary walls and wilderness field at the entrance of the village (50–100 m).	17°04'44.60"	67°37'57.00"	2,602	6/19/2008	25	12	48.0	40
	TUN 06	Catavi	Field of prickly pears adjacent to the church of the village (50–100 m).	17°04'25.20"	67°37'59.70"	2,493	6/18/2008	20	3	15.0	10
	TUN 07	Peña Colorada	Field of prickly pears outside the village (50–300 m).	17°04'24.20"	67°38'42.70"	2,543	6/18/2008	30	1	3.3	2
	CAC 02	El Calvario	Field of prickly pears adjacent to the cemetery of the village (50–150 m).	17°04'07.70"	67°39'25.50"	2,645	6/18/2008	25	5	20.0	7
	BSIA 12	Luribay	Wilderness mountainside with sparse vegetation of mainly cacti and thorny vegetation (50–200 m).	17°03'34.80"	67°39'58.40"	2,583	10/31/2008	47	11	23.4	44
	QUE 01	Achocara Bajo	Cliff of red earth along the road, forming small caves, and isolated from any village (500 m of a solitary house).	17°01'54.80"	67°40'38.60"	2,159	6/20/2008	40	5	12.5	7
	CAC 03	Palca	Field of prickly pears adjacent to some houses outside the village (20–100 m).	17°00'30.10"	67°39'25.20"	2,356	6/20/2008	49	41	83.7	263
	VIZ 02	Lacayani	Large stones at the side of the road (500 m far from an isolated house).	16°55'48.80"	67°41'32.90"	2,182	6/19/2008	39	31	79.5	284
	CAC 01	Lacayani	Wilderness valley with sparse vegetation composed mainly of cacti and thorny vegetation (500 m far from isolated houses).	16°55'33.20"	67°41'42.60"	2,210	6/18/2008	9	0	–	0
	TUN 03	Khola	Wilderness site with sparse vegetation of mainly cacti and thorny vegetation (50–500 m).	16°53'12.20"	67°42'43.10"	2,095	6/18/2008	25	7	28.0	21
	Total Rio Luribay							359	128	35.7	702
Total								646	191	29.6	978

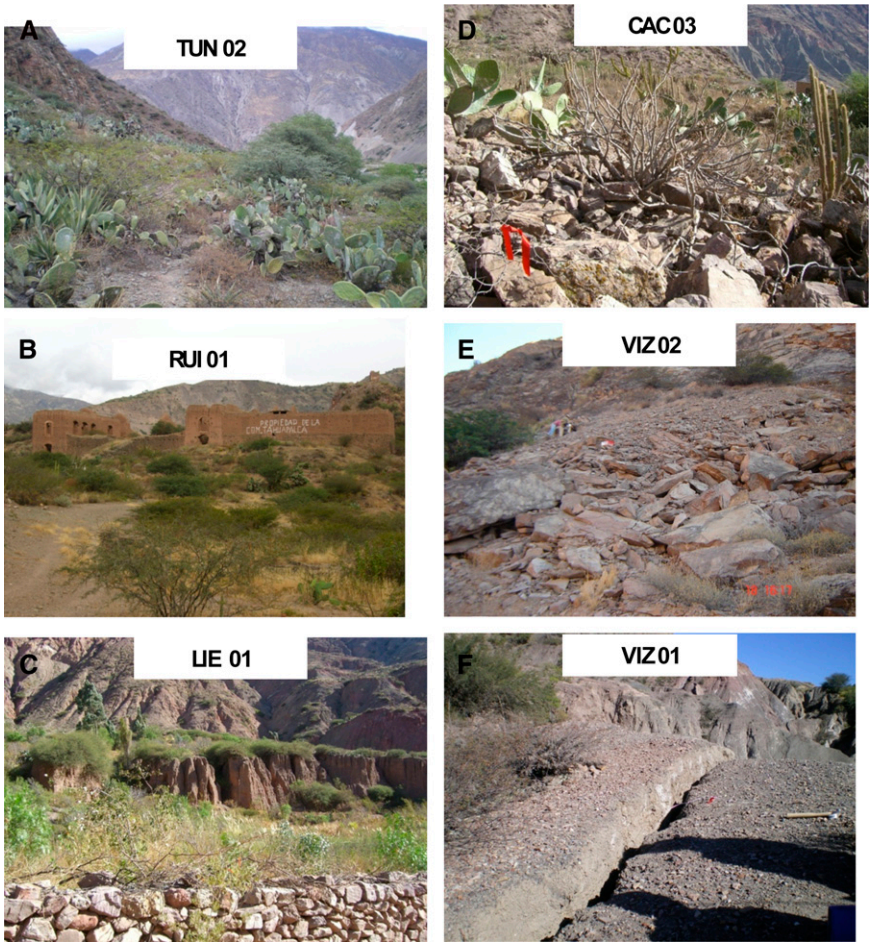

FIGURE 2. Different sampling areas. (**A**) Field of prickly pears overhanging the village of Tahuacalpa. (**B**) Ruin of 19th century barracks with mud walls next to a small cemetery on a highland overhanging the village of Tahuapalca. (**C**) Rock-pile boundary walls and wilderness field at the entrance of the village of Cuty. (**D**) Field of prickly pears adjacent to houses outside of the village of Palca. (**E**) Large stones along the driveway at 500 m from an isolated house. (**F**) Deep cracks in the ground of a small highland where vizcachas (rodents belonging to the Chinchillidae family) have been observed. This figure appears in color at www.ajtmh.org.

This fieldwork in two La Paz valleys reported here showed a wide distribution of wild populations of *T. infestans*. The sequences of cytochrome b gene of some insects confirmed the species *T. infestans*. In both valleys, wild populations were easily captured in places very near the human habitat as well as remote places, and the percentage of positive traps was similar to that reported previously in Cochabamba (~30%).[4]

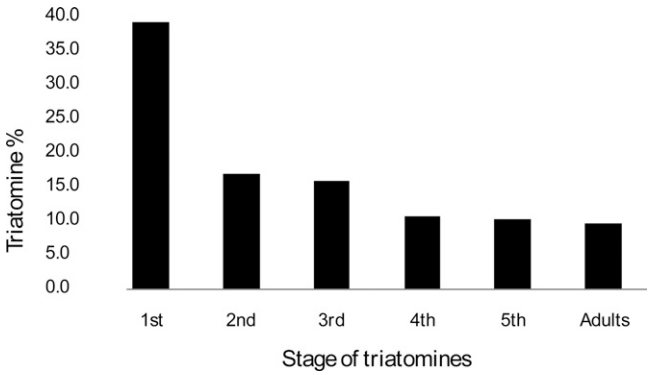

FIGURE 3. Age structure of wild *T. infestans* populations caught in the valleys of Rio La Paz and Rio Luribay.

T. infestans was found in settings never described before as habitats of this species (thorny hills, fields of prickly pear, screes, rock-pile boundary walls, ruins, and deep cracks formed by erosion). Indeed, the typical ecotope of wild *T. infestans* has been previously thought to be a rocky outcrop, except for the *T. infestans* Dark Morph found in the Gran Chaco region.[8] These new findings suggest the existence of a continuous distribution of wild populations in the valleys of La Paz. However, the distribution and abundance of these populations are little compatible with secondary wild colonization of the environment by domestic populations. However, the full distribution of *T. infestans* remains to be more accurately measured. One approach would be to investigate the different eco-regions of Bolivia where domestic *T. infestans* is endemic. Indeed, the valleys of La Paz as well as the valleys of Cochabamba, where wild populations exist, belong to the Inter-Andean Dry Forest eco-region, but the endemic area of domestic populations of *T. infestans* includes other eco regions where few data of sylvatic foci are available.

The Bolivian PNCH has expressed interest in whether or not occurrences of reinfestation by *T. infestans* have a sylvatic origin. In both valleys investigated here, the PNCH reports continuous reinfestation, and this was confirmed by local residents. Moreover, local residents reported seeing *T. infestans* in

TABLE 2
Infection rate of sylvatic *T. infestans* captured in the 17 positive areas in the department of La Paz

Area code	Number of adults		% of infected adults	Number of nymphs		% of infected nymphs	% of total infected
	Infected	Not infected		Infected	Not infected		
VIZ 01	3	0	100.0	1	2	33.3	66.6
BSIA 13	1	0	100.0	3	12	20.0	25.0
TUN 01	7	1	87.5	40	36	52.6	55.9
AGP 01	0	0	–	2	0	100.0	100.0
TUN 02	7	0	100.0	11	6	64.7	75.0
RUI 01	5	0	100.0	14	7	66.6	73.1
Total Rio La Paz	23	1	95.8	71	63	53.0	59.5
TUN 04	2	2	50.0	0	5	0.0	22.2
TUN 05	0	0	–	2	6	25.0	25.0
LIE 01	2	1	66.6	12	4	75.0	73.7
TUN 06	2	1	66.6	1	2	33.3	50.0
TUN 07	0	0	–	0	2	0.0	0.0
CAC 02	3	0	100.0	1	0	100.0	100.0
BSIA 12	8	0	100.0	6	6	50.0	70.0
QUE 01	0	0	–	1	1	50.0	50.0
CAC 03	6	3	66.6	47	72	39.5	41.4
VIZ 02	2	0	100.0	22	51	30.1	32.0
TUN 03	0	0	–	11	6	64.7	64.7
Total Rio Luribay	25	7	78.1	103	155	39.9	44.1
Total	48	8	85.7	174	218	44.4	49.5

the sylvatic environment and flying to dwellings. These data suggested the capacity of the bug to fly from their natural habitats to human-developed areas. Moreover, our work points to the utility of adding interviews with locals to help determine the presence of sylvatic triatomines: information from our interviewees was strongly corroborated in the field. To effectively assess the epidemiological role of wild *T. infestans* populations, it is necessary to better understand the mechanisms of the reinfestation and to discriminate residual populations, which can develop after insecticide spraying, from reinfestation by sylvatic populations. Therefore, population genetic analyses by different genetic markers should be useful. Also, it is necessary to bring eco-epidemiological research to understand the anthropological, sociological, and biological determinants of reinfestation.[14,15]

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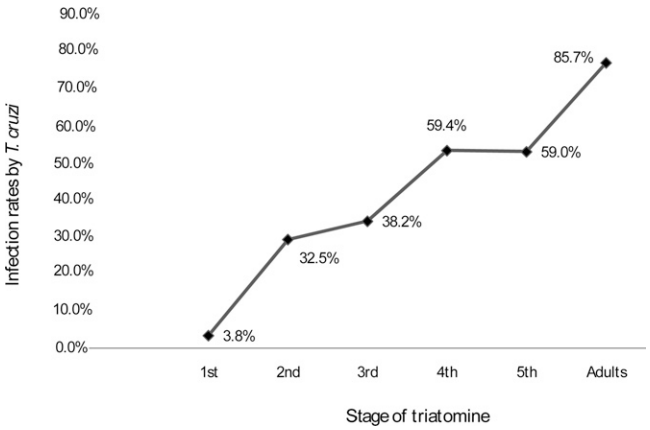

FIGURE 4. Infection rate of the different stages of wild *T. infestans* caught in the valleys of Rio La Paz and Rio Luribay.

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