Prevalence of *Trypanosoma cruzi* and Other Trypanosomatids in Frequently-Hunted Wild Mammals from the Peruvian Amazon

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Abstract. To better understand the ecology of *Trypanosoma cruzi* in the northeastern Peruvian Amazon, we evaluated the prevalence of *T. cruzi* and other trypanosomatids in four orders of wild mammals hunted and consumed by inhabitants of three remote indigenous communities in the Peruvian Amazon. Of 300 wild mammals sampled, 115 (38.3%) were infected with trypanosomatids and 15 (5.0%) with *T. cruzi*. The prevalence of *T. cruzi* within each species was as follows: large rodents (*Cuniculus paca*, 5.5%; *Dasyprocta* spp., 2.6%), edentates (*Dasypus novemcinctus*, 4.2%), and carnivores with higher prevalence (*Nasua nasua*, 18.8%). The high prevalence of *T. cruzi* and other trypanosomatids in frequently hunted wild mammals suggests a sizeable *T. cruzi* sylvatic reservoir in remote Amazonian locations.

Because of its high prevalence, and occurrence in 21 countries, Chagas disease is one of the most important, neglected tropical diseases in Latin America.¹ It is estimated that between 6 and 8 million people are infected in Latin America, of whom 12,000 die of the disease each year.¹ The Amazon Basin, inhabited by 30 million people, is the largest tropical biome in which both animal reservoirs and multiple species of triatomine vectors of *Trypanosoma cruzi* coexist, representing a major threat to human populations.²

About 33 species in six mammalian genera act as reservoirs for *T. cruzi* in the Amazon, including marsupials, bats, rodents, edentates, carnivores, and primates.³ However, the challenges of studying wildlife populations in remote areas have limited the identification of natural hosts of *T. cruzi* in the Peruvian Amazon, and only three are previously documented: *Saimiri boliviensis*, *Saguinus nigricollis* and *Didelphis paraguayensis pernigra*,⁴ whereas more are described from regions of the Brazilian Amazon.^{2,5}

Chagas disease is mainly transmitted by contact with feces from infected triatomine insects.⁴ Oral transmission through food and beverages contaminated with such vector feces has also been documented and may be more important than previously considered, accounting for up to 70% of acute cases in the Brazilian Amazon.⁶ Oral transmission through the consumption of undercooked meat may also occur, but has not been conclusively confirmed.^{7–9} As hunting is one of the main subsistence activities in the Amazon Basin, local villagers might be at the risk of infection during excursions into the forest. This study evaluated the prevalence of *T. cruzi* and other trypanosomatids in blood samples from four taxonomic orders of wild mammals hunted and consumed by the inhabitants of rural communities in the Peruvian Amazon.

We studied a sample of 300 wild mammals from 13 species and four taxonomical orders that had been hunted for subsistence and household income by local villagers in three

remote indigenous communities in the Peruvian Amazon from 2009 to 2011 (Table 1, Figure 1). No sampled animals were killed exclusively for this study. Blood samples were directly spotted onto Whatman paper during butchering by trained local villagers, preserved on silica gel desiccant, transported to the US Naval Medical Research Unit No. 6 in Lima, and stored at –80°C. The research protocol was approved by the Dirección General de Flora y Fauna Silvestre (0350-2012-AG-DGFFS-DGEFFS) of Peru.

We isolated DNA from samples using the QIAamp DNA mini kit (QIAGEN, Valencia, CA). DNA concentrations were quantified using the NanoDrop-1000 spectrophotometer (Thermo Scientific, Somerset, NJ). We conducted a polymerase chain reaction (PCR) (Applied Biosystems) targeting the 24S alpha subunit rRNA gene of trypanosomatids overall using primers D75 and D76. Subsequently, a nested-PCR targeted an internal *T. cruzi*-specific region of the same gene using primers D71 and D72.¹⁰ Ultrapure water, *Leishmania* (V.) *brasiliensis* and *T. cruzi* (Y and Tulahuen strains) were used as controls (Figure 2). Between 2009 and 2011, hunting records of eight out of 60 families from Nueva Esperanza were used to estimate the annual number of species hunted and consumed by local inhabitants and assess the potential consumption of *T. cruzi*-infected mammals in these communities. The prevalence of *T. cruzi* and trypanosomatids between orders and communities were compared using χ^2 test and Fisher's exact test according to the sample size. Confidence intervals of 95% were also estimated accordingly using RStudio version 0.98.1091 (The R Foundation for Statistical Computing, Vienna, Austria).

Of 300 sampled mammals, 115 (38.3%; 95% CI = 32.8–44.1) were infected with trypanosomatids and 15 (5.0%; 95% CI = 2.8–8.1) of the latter with *T. cruzi* (Table 1). Trypanosomatids were identified at all sampling sites with different prevalence ($P = 0.013$): Nueva Esperanza (41.1%, 109/265), Diamante/7 de Julio (27.3%; 3/11), and Sol Naciente (12.5%; 3/24), whereas all *T. cruzi*-positive samples came exclusively from Nueva Esperanza (5.7%; 15/265).

The order Rodentia showed the highest prevalence of trypanosomatids (79/167; 47.3%), followed by Artiodactyla (23/74; 31.1%), Carnivora (9/34; 26.5%), and Edentata (4/25; 16.0%). *Trypanosoma cruzi* was found in all taxonomic orders

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TABLE 1

Prevalence of *Trypanosoma cruzi* and trypanosomatids in four orders and 13 animal species from three rural communities of the Peruvian Amazon, 2009–2011

Order	Species	N	Trypanosomatids*			<i>Trypanosoma cruzi</i>		
			Positives*	%	95% CI†	Positives	%	95% CI†
Rodentia	<i>Cuniculus paca</i>	128	75	58.6	49.6–67.2	7	5.5	2.2–10.9
	<i>Dasyprocta</i> spp.	38	4	10.5	2.9–24.8	1	2.6	0.00–13.8
	<i>Sciurus igniventris</i>	1	0	0.0	0.0–97.5	0	0.0	0.0–97.5
	Subtotal	167	79	47.3	39.5–55.2	8	4.8	2.1–9.2
Artiodactyla	<i>Tapirus terrestris</i>	9	5	55.6	21.2–86.3	0	0.0	0.0–33.6
	<i>Tayassu pecari</i>	4	2	50.0	6.8–93.2	0	0.0	0.0–60.2
	<i>Mazama americana</i>	15	6	40.0	16.3–67.7	0	0.0	0.0–21.8
	<i>Tayassu tajacu</i>	44	10	22.7	11.5–37.8	0	0.0	0.0–8.0
	<i>Mazama gouazoubira</i>	2	0	0.0	0.0–84.2	0	0.0	0.0–84.2
	Subtotal	74	23	31.1	20.8–42.9	0	0.0	0.0–4.9
Carnivora	<i>Nasua nasua</i>	32	9	28.1	13.7–46.7	6	18.8	7.2–36.4
	<i>Puma concolor</i>	1	0	0.0	0.0–97.5	0	0.0	0.0–97.5
	<i>Leopardus pardalis</i>	1	0	0.0	0.0–97.5	0	0.0	0.0–97.5
	Subtotal	34	9	26.5	12.9–44.4	6	17.6	6.8–34.5
Edentata (Xenarthra)	<i>Dasybus novemcinctus</i>	24	4	16.7	4.7–37.4	1	4.2	0.1–21.1
	<i>Priodontes maximus</i>	1	0	0.0	0.0–97.5	0	0.0	0.0–97.5
	Subtotal	25	4	16.0	4.5–36.1	1	4.0	0.1–20.4
Total		300	115	38.3	32.8–44.1	15	5.0	2.8–8.1

There were statistically significant differences between the four orders studied in the prevalence of *T. cruzi* ($P = 0.003$) and trypanosomatids ($P = 0.002$).

* Trypanosomatids including *T. cruzi*.

† 95% confidence interval was computed using the binomial test.

except Artiodactyla, with the rodent *Cuniculus paca* (7/128; 5.5%) and the carnivore *Nasua nasua* (6/32; 18.8%) accounting for 46.7% and 40.0% of cases, respectively. The small number of *C. paca* ($N = 11$) and *N. nasua* ($N = 1$) sampled in Diamante/7 de Julio and Sol Naciente may explain the absence of *T. cruzi* in these communities.

Eight families from Nueva Esperanza reported hunting 739 animals over 3 years. We estimated a yearly harvest of 31

animals per family, and 1,847 animals for the whole community of approximately 307 inhabitants in 60 families. Considering only species that were *T. cruzi*-positive in our study, we estimated that 45 infected animals might be consumed annually in the community: *C. paca* ($N = 24$), *N. nasua* ($N = 17$), *Dasybus novemcinctus* ($N = 2$), and *Dasyprocta* spp. ($N = 2$).

Trypanosoma cruzi was found in all sampled orders (Rodentia, Carnivora, and Edentata), except Artiodactyla, and in four of

FIGURE 1. Location of the three rural communities in the northeastern Peruvian Amazon from where samples were collected: Nueva Esperanza (04°19'53" S; 71°57'33" W) in the Yavari-Mirin basin, Sol Naciente (03°38'26" S, 73°12'57" W) near the Amazon river, and Diamante/7 de Julio (04°36'43" S, 72°46'12" W) in the Tamshiyacu-Tahuayo Regional Conservation Area.

FIGURE 2. Identification of trypanosomatids and *Trypanosoma cruzi* by nested polymerase chain reaction in 14 wild mammals only for illustration purposes. (A) PCR for the identification of trypanosomatids (225–265 bp). Lane 1: negative control (water). Lane 2: positive control #1 (*Trypanosoma cruzi*, Y strain – 265 bp). Lane 3: positive control #2 (*Trypanosoma cruzi*, Tulahuen strain – 250 bp). Lane 4: + control (*Leishmania (V.) braziliensis* – 225 bp). Lane 5, 7–16, 18: positive samples (double bands in lanes 7 and 14 may depict multiple infections with trypanosomatids). Lane 6, 17: negative samples. (B) Nested-PCR for the identification of *Trypanosoma cruzi* (110–125 bp). Lane 1: negative control (water). Lane 2: positive control #1 (*Trypanosoma cruzi*, Y strain – 125 bp). Lane 3: positive control #2 (*Trypanosoma cruzi*, Tulahuen strain – 110 bp). Lane 4: negative control (*Leishmania (V.) braziliensis*). Lane 5, 7, 9, 10: positive samples. Lane 6, 8, 11–18: negative samples.

seven species with five or more specimens tested, suggesting a high prevalence of *T. cruzi* in frequently hunted mammals. Coatis (*N. nasua*) showed the highest prevalence of *T. cruzi*, confirming the important role of this carnivore in the sylvatic cycle of Chagas disease in the Peruvian Amazon and consistent with evidence from Brazil.^{11,12} It has been proposed that coatis may become infected through oral transmission rather than vectorial transmission by ingesting contaminated triatomines and feeding on small mammals.¹² We also found *T. cruzi* (4.2%) in armadillos (*Dasypus novemcinctus*), which are well-known reservoirs of *T. cruzi* in both peridomestic and sylvatic cycles.¹³ Finally, we found *T. cruzi* naturally infecting large rodents (*C. paca*, 5.5%, and *Dasyprocta* spp., 2.6%), which is also consistent with the findings in the Brazilian Amazon.^{14,15} Although epidemiological studies on large rodents are limited, they are considered ancient hosts of *T. cruzi*, and may be effective reservoirs.¹⁶ The large rodents *C. paca* and *Dasyprocta* spp. are unlikely to be predated by coatis, but are predated by larger carnivores (e.g., felines), which in turn could possibly become infected. The peccaries *Tayassu tajacu* and *Tajassu pecari* have also been suggested as reservoirs of *T. cruzi* in a previous study,¹² however, we found no peccary infected. Our study in remote and isolated communities shows a 5.0% prevalence of *T. cruzi* in four species of large rodents, carnivores, and edentates. Although the high natural prevalence of *T. cruzi* infections in frequently hunted wild mammals might suggest a large *T. cruzi* sylvatic reservoir in the Peruvian Amazon, we cannot rule out the possibility that the disease could make these mammals more vulnerable to be hunted, and thus the reported prevalence might be overestimating the true prevalence in the Amazon.

The high prevalence of trypanosomatids (38.3%) found in all sampled mammalian orders suggests that several parasites of

this family in remote Amazonian regions may be widely distributed across different mammalian groups rather than being confined to specific hosts. We cannot exclude that pre-infection with other trypanosomatids might increase the susceptibility to *T. cruzi* infection in these mammals as previously suggested for *Trypanosoma lewisi* and *Leishmania chagasi*.¹⁷

Dogs may also play an important role in the peridomestic cycle of Chagas disease as potential reservoirs and may be accurate sentinels for identifying areas of active *T. cruzi* transmission.^{6,18} Although we did not sample domestic dogs in this study, they may help to establish a link between the peridomestic and sylvatic cycles of *T. cruzi* in rural communities as dogs usually accompany hunters on foot during excursions into the forest and can be vectorially infected. In addition, dogs in these communities are often fed raw viscera and oral transmission cannot be ruled out.

Evidence of oral transmission of *T. cruzi* through consumption of undercooked meat is currently inconclusive, but its occurrence in the Amazon basin is suspected,⁸ and to our knowledge, no study has rejected this hypothesis. The consumption of bushmeat in our sampled communities was typical of bushmeat consumption across Amazon rural communities, in which wild meat represents an important animal protein source. An estimated 113,000 wild animals are hunted annually in the department of Loreto.¹⁹ We estimated that the study community annually hunts and eats 45 animals infected with *T. cruzi*, translating to 0.75 infected animals typically consumed by a large extended family per year, not accounting for sharing of meat between families, suggesting recurring opportunities of infection.¹⁸ Further studies addressing a relationship between wild meat consumption and Chagas disease are required to better understand the risk of infection in Amazonian communities.

In Peru, 830 human cases of *T. cruzi* were reported from 2004 to 2015,²⁰ most of them (81.3%) in the Peruvian southern highlands, in the department of Arequipa. Only 23 cases were documented in Loreto, Peruvian Amazon's largest department in urban and rural population, which probably greatly underestimates the burden of *T. cruzi* infection, because of the absence of routine diagnosis and limited access to healthcare in remote indigenous communities.

Received January 12, 2017. Accepted for publication June 30, 2017.

Published online August 28, 2017.

Acknowledgments: We thank the residents of Nueva Esperanza (Yavari-Mirin River), Sol Naciente (Amazon River), and Diamante/7 de Julio (Tamshiyacu-Tahuayo Community Reserve) who actively participated in sample collection.

Financial support: This work was been supported by LA Zoo, and the training grant 2D43 TW007393 awarded to AGL by the Fogarty International Center of the U.S. National Institutes of Health; and the Earthwatch Institute. The sponsors had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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