

SHORT REPORT: *TRYPANOSOMA CRUZI* DETECTION IN BLOOD BY XENODIAGNOSIS AND POLYMERASE CHAIN REACTION IN THE WILD RODENT *OCTODON DEGUS*

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Abstract. We detected *Trypanosoma cruzi* in blood samples of the wild rodent *Octodon degus* by xenodiagnosis and a polymerase chain reaction (PCR) using the domestic and wild vectors of Chagas disease, *Triatoma infestans* and *Mepraia spinolai*, respectively. We captured 35 rodents and extracted DNA from blood samples and intestinal contents of vectors fed on *O. degus*. Our results indicate that the percentage of rodents naturally infected with *T. cruzi* depends on the biologic sample used for PCR and on the vector species for xenodiagnosis. The PCR with blood samples did not detect *T. cruzi* DNA, but the PCR with intestinal contents showed that both vectors were positive for *T. cruzi*. The PCR performed with *M. spinolai* intestinal contents detected four times more *T. cruzi*-positive *O. degus* than the PCR with *Triatoma infestans* intestinal contents (22.9% and 5.7%, respectively). We report the improvement of *T. cruzi* detection in sylvatic animals by a combination of PCR and xenodiagnosis using sylvatic vectors, especially in disease-endemic areas with low parasitemias in mammals.

Chagas disease is a serious human parasitic disease in the Americas that is caused by the flagellate protozoan *Trypanosoma cruzi* and transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera: Reduviidae).¹ Because of low parasitemia in different hosts with chronic infections, the diagnosis of this disease is made mainly by serologic methods. However, low sensitivity of currently used parasite detection techniques such as xenodiagnosis limit their use in the diagnosis of Chagas disease. To avoid this problem, efforts have been made to detect *T. cruzi* DNA in blood samples by polymerase chain reaction (PCR) assays. Specific amplification of kinetoplast minicircle DNA from animals and humans has been reported.^{2,3} Detection of *T. cruzi* minicircle DNA by PCR also has been used in naturally infected triatomines^{4,5} and insect vectors fed on blood from infected patients.^{6,7}

The main vector of Chagas disease in Chile is the predominantly domestic bug *Triatoma infestans*, which has been successfully controlled in this country.⁸ The wild vector *Mepraia spinolai* is often found in rocky sylvatic ecotopes associated with small rodents and rabbits and also in peridomestic ecotopes such as henhouses and goat corrals.⁹ *Mepraia spinolai* is endemic in Chile and distributed between the 22°S and 33°S.¹⁰ Unlike other triatomines, this species is usually a diurnal bug, but it is active at night.¹¹

More than 150 species of sylvatic mammals have been found naturally infected with *T. cruzi*, and some of these reservoirs have an important role in the maintenance and interaction of domestic, peridomestic, and sylvatic cycles of Chagas disease.¹² One study in Chile with the native rodent *Octodon degus* and microscopic blood examination and xenodiagnosis with *Triatoma infestans* reported an infection rate of 8.9%.¹³ Other studies detected variable frequencies of infection ranging from 2% to 21% by xenodiagnosis with *Triatoma infestans* and hemoagglutination, respectively.^{14,15} *Octodon degus* inhabits stone quarries throughout the year,

which makes it an ideal host for *M. spinolai*. In this study, we analyzed detection of *T. cruzi* by PCR in *O. degus* blood samples and intestinal contents of both insect vectors, *Triatoma infestans* and *M. spinolai* fed on peripheral blood (xenodiagnosis) of *O. degus*.

The study site was located in the Las Chinchillas National Reserve (31°30'03"S, 71°06'20"W), which is approximately 300 km north of Santiago, Chile and 60 km east of the Pacific coast. The reserve is in a mountainous terrain (area = 4,229 hectares) with a semiarid climate and sporadic precipitation concentrated during winter months.¹⁶

In this study, we captured 35 *O. degus* with traps (H. B. Sherman Traps, Tallahassee, FL) in diurnal and nocturnal periods. Captured animals were weighed and anesthetized with isofurane at a dose of 13 mg/kg of body weight. A small area of abdominal skin was cleaned for anesthetic injection. Once the animal was completely anesthetized, 0.5–1.0 mL of blood was withdrawn by cardiac venipuncture using heparinized syringes. After blood samples were obtained, *O. degus* were transported to the laboratory and kept in a breeding ground (Faculty of Medicine, University of Chile, Santiago, Chile) at 25°C and a relative humidity of 60% with a 12:12 hour light:dark photoperiod, individually housed in plastic cages (31.0 × 37.0 cm), and given water and alfalfa pellets *ad libitum*.

Extraction of genomic DNA from 0.2 mL of whole blood from *O. degus* was performed with the E.Z.N.A. kit (Omega Biotek, Inc., Doraville, GA). DNA was concentrated by ethanol precipitation and resuspended in 50 µL of deionized, sterile water. The PCR was performed as previously reported using primers 121 and 122 to amplify the variable region of minicircle kinetoplast DNA.^{17,18} The sensitivity of detection of minicircle DNA by PCR with the primers and conditions used is 0.4 log parasite genome equivalents/mL.¹⁹ Each experiment included positive and negative controls. Samples were tested in triplicate, and an animal was considered positive when at least two of the three assays showed positive results. Doubtful samples (i.e., when one of three PCR assays showed positive results) were retested four times by PCR. Animals with four or more positive PCR results were consid-

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ered positive but with low parasitemias. The PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. A 330-basepair product indicated a positive result.

For xenodiagnosis purposes, *O. degus* specimens were intramuscularly anesthetized with 50 mg/kg of ketamine and placed in a tray with 10–20 uninfected first and second instar nymphs of each vector species. *Triatoma infestans* and *M. spinolai* nymphs were obtained from established insect colonies at the Faculty of Medicine and Faculty of Science of the University of Chile (Santiago, Chile), respectively. The amount of blood extracted by each pool of bugs from *O. degus* did not differ between the two vector species (*t* test; $F_{1,14} = 0.155$, $P = 0.699$). Thirty and sixty days post-feeding, a pool of feces from all bugs fed with each *O. degus* was obtained by abdominal extrusion. Five microliters of fresh fecal material was compressed between a glass slide and an 18 mm × 18 mm coverslip²⁰ and examined with a light microscope (Diaphot-FXA; Nikon, Tokyo, Japan). Motile parasites in 50 microscopic fields were observed at a magnification of 400×. A pool of total intestinal contents 60 days post-feeding was mixed with 200 μL of phosphate-buffered saline, centrifuged at 10,000 × *g*, and frozen at –20°C for PCR assay. A 1–5-μL sample of this extract was boiled for 10 minutes and used as a DNA template. Since intestinal contents were free of fresh blood, no DNA extraction was required.⁵ After xenodiagnosis, all animals were returned to the study site. Experiments were reviewed and approved by the Ethical Committee of the Faculty of Medicine, University of Chile.

Our results indicated that 22.9% of the *O. degus* at the study site were naturally infected with *T. cruzi*. However, these results varied with the biologic sample used for the PCR and the vector species used for the xenodiagnosis. Specifically, PCR analysis of blood samples did not detect *T. cruzi* DNA in the *O. degus* specimens. Conversely, results of the PCR of intestinal contents indicated that both vectors were positive for *T. cruzi*. The PCR performed with *M. spinolai* intestinal contents detected four times more *T. cruzi*-positive *O. degus* than the PCR with *Triatoma infestans* intestinal contents (22.9% and 5.7%, respectively). These differences in detection sensitivity were statistically significant ($\chi^2 = 4.20$, degrees of freedom = 1, $P = 0.040$). Table 1 summarizes detection of *T. cruzi* by each vector species using blood and intestinal content samples for PCR analysis. Direct microscopic observation of fecal samples from both vector species showed that two *O. degus* were infected with *T. cruzi* (5.7%). These rodents were also positive for *T. cruzi* by PCR for intestinal contents of both vector species.

The PCR assay used for parasitologic diagnosis is highly

sensitive because the target DNA is present in high copy numbers. This method has been useful with blood samples in detecting *T. cruzi*-infected animals.² However, in our study, this method was not sensitive enough to detect positive *O. degus* rodents by using blood samples. Results of PCR assays performed on intestinal contents of insect vectors fed on *O. degus* blood indicated that some were infected with *T. cruzi*. The percentage of infected rodents was dependent on the vector species used for xenodiagnosis. We suggest that *M. spinolai* is better than *T. infestans* as insect vector in propagating *T. cruzi* circulating in the wild rodent *O. degus*. This is probably the result of a vector–parasite-specific coevolutionary process. In an optimal *T. cruzi*-vector system, parasite and vector originated from the same locality.²¹

Our results of higher parasite detection sensitivities by xenodiagnosis combined with intestinal content PCR assays when compared with blood sample PCRs should be further analyzed. Although the volume of blood used to extract DNA and the volume to feed insect vectors was equivalent, parasitemia was observed only with insect intestinal samples. This finding can be explained by the high rate of parasite amplification of epimastigote forms in the intestines of the bugs. Parasitemias in mammals during the chronic phase of the disease may be too low to be detected by the PCR. Parasitemia varies among *O. degus*, and rodents that were positive by PCR with both insects showed higher parasitemias than those detected by PCR and only *M. spinolai* samples. This fact is confirmed by the higher amounts of DNA amplicons in four rodents. Conversely, four *O. degus* were detected only with *M. spinolai* and seven PCR assays.

Lower infection rates in *O. degus* were observed in the present study when compared with results of previous surveys. The infection rate detected in this study (22.9%) is less than half the rate reported for this rodent species in Las Chinchillas National Reserve five years ago.² Both studies used PCR assays on *O. degus* blood samples. However, our results with blood did not detect *T. cruzi*-infected rodents. This fluctuation in the percentage of *T. cruzi*-infected *O. degus* may have resulted from natural oscillations in parasite-vector-host systems.

In summary, this study reports the improvement of *T. cruzi* detection in sylvatic animals by a combination of PCR and xenodiagnosis using sylvatic vectors from the same area as the mammals analyzed. We showed that using *M. spinolai*, the wild vector of Chagas disease in Chile, it would be possible to carry out more accurate epidemiologic studies in disease-endemic areas of with low *T. cruzi* parasitemias.

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TABLE 1

Percentage of *Octodon degus* infected with *Trypanosoma cruzi* using blood and intestinal content as DNA samples for polymerase chain reaction (PCR) and two vector species for xenodiagnosis

DNA sample for PCR	<i>T. cruzi</i> -infected <i>O. degus</i> (%)	
	<i>Triatoma infestans</i>	<i>Mepraia spinolai</i>
<i>Octodon degus</i> blood	0/35 (0)	0/35 (0)
Bug intestinal content	2/35 (5.71)	8/35 (22.86)

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