SHORT REPORT: *TRYPANOSOMA CRUZI* INFECTION IN WILD MAMMALS FROM A CHAGASIC AREA OF CHILE

MARLENE ROZAS, CAREZZA BOTTO-MAHAN,* XIMENA CORONADO, SYLVIA ORTIZ, PEDRO E. CATTAN, AND ALDO SOLARI

Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile; Department of Ecological Sciences, Faculty of Sciences, University of Chile, Santiago, Chile; Department of Biological Sciences, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile

Abstract. We report results of PCR-DNA based detection of *Trypanosoma cruzi* in wild mammal reservoirs from a chagasic area of Chile. We analyzed 157 blood samples from wild mammals including the marsupial *Thylamis elegans* and the rodents *Octodon degus*, *Phyllotis darwini,* and *Abrothrix olivaceus*. In addition, 42 blood samples from goats (i.e., a peridomestic mammal) were analyzed. Blood samples were used to extract DNA, and PCR was performed using the amplification of minicircle DNA sequences. Southern analysis was used to confirm diagnosis with a universal probe of P^{32}-labeled kinetoplast DNA. Altogether, 51% of the wild and 36% of the peridomestic mammals were infected with *T. cruzi*. These findings suggest that the real *T. cruzi* infection levels in wild and peridomiciliary reservoirs are higher than those previously determined with serological and parasitological conventional methods. The relevance of our results is discussed in term of the risk factor of infection in human.

Chagasic infection has been detected in rural and suburban areas of Chile, between 18°30′ and 34°16′ S corresponding to arid and semiarid zones. Currently in Chile, 142,000 people are infected and another 1,000,000 are at risk of infection.[1] In the domestic cycle, the main vector of human Chagas disease is *Triatoma infestans*. In Chile, this vector has been reduced exhaustively by means of periodic spraying in human dwellings and peridomestic habitats from endemic areas, according to the South Cone initiative to eliminate *T. infestans.*[1] In our country, the prevalence of Chagas disease in children dropped substantially in the past decade, as well as the infestation rates of human dwellings.[2]

Mepraia spinolai is an endemic Chilean vector that inhabits wild and peridomestic habitats such as wild rodent burrows, chicken houses, and goat corrals. This species distributes from 26° to 33° S, selecting sunny and sheltered places from the mountains to the sea border.[3] It feeds at daylight by painless punctures on wild animals; however, it can approach human dwellings to feed from peridomestic animals and human beings. Its index of infection with *T. cruzi* can reach up to 70% by PCR assays in some northern areas (Botto-Mahan C and others, unpublished data), and its human blood index reaches 7.4%.[4] Considering its alimentary profile (i.e., the blood host sources), this species is in a intermediate position between domestic-peridomestic species (Type II and III), indicating that even though is mainly a wild species its approach to human environments turns it in a potentially dangerous vector for *T. cruzi* transmission.[5]

In America, more than 150 species of wild mammals have been found naturally infected with *T. cruzi,* and some of these reservoirs possess an important role in the maintenance and interaction of domestic, peridomestic, and wild cycles of Chagas disease.[6,7] In Chile, several studies have determined the presence of *T. cruzi* in domestic and peridomestic mammals.[8] *Capra hircus* is one of the most studied peridomestic mammals with infection percentages between 4.5% and 18.0% by xenodiagnosis.[9] However, scarce information has been reported about *T. cruzi* infection in wild mammals. The first study using microscopic blood examination and xenodiagnosis detected 8.9% of infection in the rodent *Octodon degus.*[10] Other studies in *O. degus* revealed 2.2% and 21.4% of infection by xenodiagnosis and hemoagglutination, respectively.[11,12] The rodent *Phyllotis darwini* has also been reported infected with *T. cruzi,* 8–10% by xenodiagnosis.[11,12]

Chagas disease diagnosis in animals has been performed mainly by serological or parasitological methods such as xenodiagnosis, which are highly specific but of limited sensitivity. Estimates indicate that only 20–50% of infected human beings are detected by these methods, implying a high amount of false negatives.[13] PCR assay has proven to be sensitive and specific in human blood samples.[14] Here, we amplify the minicircle variable regions by means of oligonucleotides specific for the conserved regions present in all the minicircles of *T. cruzi.*[14] The goal of the study is to assess the possible role that *M. spinolai* might have in the persistence of *T. cruzi* in northern Chile by looking for wild mammal reservoirs of Chagas disease.[11] The close association between some mammal reservoirs and human dwellings in endemic zones makes this study essential to evaluate the risk factors for human infections.

The study site is located at Las Chinchillas National Reserve (31°30'03″ S, 71°06'20″ W), about 300 km north from Santiago (Chile) and 60 km east from the Pacific coast. The reserve is in a mountainous terrain of 4,229 ha. The climate is semiarid, with sporadic precipitation concentrated during austral winter months (June through August). The vegetation is thorn scrub composed mainly of spiny dicots, bromeliads, and cacti, depending on sun exposure. Mainly wild mammals, such as *Phyllotis darwini*, *Octodon degus*, *Abrothrix olivaceus*, *Olygorizomis longicaudatus,* and *Thylamys elegans,* inhabit the study area, however, some traffic of cattle yard animals such as goats (*Capra hircus*), horses, and cattle is observed.

In this study, 157 wild mammal blood samples from 4 species (*P. darwini*, *O. degus*, *A. olivaceus*, and *T. elegans*) were examined. In addition, 42 goat blood samples were included for comparative purposes. Wild mammals were captured with Sherman traps in diurnal and nocturnal periods during summer. Captured animals were weighed and anesthetized with isofurane at a dose of 13 mg/kg body weight. A small area of abdominal skin was cleaned for anesthetic injection. Once the

* Address correspondence to Carezza Botto-Mahan, Department of Ecological Sciences, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile. E-mail: cbotto@uchile.cl

animal was completely anesthetized, 0.5 to 1.0 mL of blood was withdrawn by cardiac venopuncture using heparinized tuberculin syringes. After recovering from the anesthesia, animals were released to the capturing area. Blood collection from goats was performed by jugular vein puncture from a previously cleaned skin area. Blood samples were stored at –20°C until molecular analysis.

The DNA was extracted with the E.Z.N.A kit (Omega Bioteck, Inc., Doraville, GA), allowing the isolation of genomic DNA from whole blood. Finally, DNA was concentrated by ethanol precipitation and further resuspension in 50 μL of deionized sterile water. PCR was performed as previously reported, using primers 121 and 122 directed to amplify the hypervariable region of minicircle kinetoplast DNA.[14,15] Each run included positive and negative controls. Samples were tested in triplicate, and an animal was considered positive when the three assays turned out positives. PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. A 330 base pairs (bp) product represents a positive assay.

For confirmation, the Southern analysis was performed with 10 μL of each PCR assay that was electrophoresed, transferred onto Hybond N+ nylon membranes (Amersham, Buckinghamshire, UK), and cross-linked by ultraviolet light for DNA fixation. After transferring PCR products, membranes were prehybridized for at least 2 hours at 55°C and finally hybridized with total kinetoplast DNA labeled with P^{32} (1×10^6 cpm/membrane). After hybridization, membranes were washed three times for 30 minutes each with 2 × SSC, 0.1% SDS at 55°C, and exposed later in the Molecular Imager FX (Bio-Rad Laboratory, Hercules, CA).

Results for wild mammals indicated that 43.3% were positives ($N = 68$), having all the species similar infection levels. Meantime, results from *Capra hircus* indicated that 26.2% were positives ($N = 11$). Additional positives were detected by Southern analysis in samples that resulted PCR positive in only one or two of the three PCR assays. In wild mammals, 51.0% ($N = 80$) were positives with the radioactive probe, and 35.7% goats ($N = 15$) were positives. The greatest difference comparing infected animals with both methods is observed in *A. olivaceus*, where the positive cases increased from 45.5% to 59.1%.

It is possible to observe based on the reported results that the percentage of positive cases increased when PCR and hybridization results are combined. Table 1 shows the results by species for both techniques. The percentage of positive amplification by PCR was of 39.7% considering all samples, whereas using PCR and hybridization assays the percentage rose to 47.7%. The difference between PCR and Southern analysis results might reflect the very low parasitemia in approximately 8–10% of the animals, which are detected positive by the hybridization signal, but not by ethydium bromide staining. However, statistical analysis did not show significant differences between the methods ($\chi^2_1 = 2.30$, $P = 0.130$).

TABLE 1

Percentage of *Trypanosoma cruzi* infection in peridomestic and wild mammals by PCR assays

Reservoir species	Cycle	Infected out of total cases (%)	
		PCR (ethidium bromide staining)	PCR and southern analysis
Capra hircus (goat)	Domestic	11/42 (26)	15/42 (36)
Thylamis elegans (marsupial)	Wild	6/13 (46)	6/13 (46)
Octodon degus (rodent)	Wild	19/45 (42)	23/45 (51)
Phyllotis darwini (rodent)	Wild	23/55 (41)	25/55 (45)
Abrotrix olivaceus (rodent)	Wild	20/44 (45)	26/44 (59)

Assessments of *T. cruzi* infection levels in mammal reservoirs from the wild cycle of Chagas disease in Chile are scarce. This is the first study to report *T. cruzi* infection in mammal reservoirs with a highly sensitive methods as PCR and hybridization test. The infection level in wild mammals was 51%, indicating that they might be playing an important role as reservoirs of *T. cruzi*. Previous results for these species from the same endemic area reported much lower infection levels by hemoagglutination and xenodiagnosis.[11,12] Even though peridomestic animals showed lower infection level than wild ones (36%), only marginally significant differences were detected ($\chi^2_1 = 3.13$, $P = 0.077$).

The difference between our molecular results and those serological results published in the literature can be explained by the low sensitivity of the conventional hemoagglutination technique, which would explain those false negative cases, and the same as for xenodiagnosis results. Our results also indicate that the *T. cruzi* infection levels in wild and peridomestic mammals are higher than those previously reported.

The relevance of this study is that peridomestic mammals such as goats are often found in close contact with humans as well as associated to wild habitats when grazing. This fact can play an important role in the permanence of *T. cruzi* in the domestic cycle and in the interaction between the wild and domestic cycles. The high number of infected wild mammals in the study area can be explained by the high level of infection in *M. spinolai* (Botto-Mahan C and others, unpublished data). The human participation or its blood index in the diet of *M. spinolai* is close to 7.4%, and for other domestic and peridomiciliary animals like cats, dogs, goats, and rabbits, figures range from 3.7% to 53.1%,[4] suggesting this vector is at least partially involved in the domestic cycle.

In Chile, human dwelling and peridomiciliary infestation by the domestic vector *Triatoma infestans* has been controlled. However, the threat that *M. spinolai* can colonize human dwellings, same as reported for other secondary vectors in different endemic areas of South America, is a tangible possibility.[16] An understanding of *T. cruzi* transmission throughout endemic areas of Chile will allow health care workers to assess the risk factors associated with exposure to American trypanosomiasis. Future molecular epidemiologic studies should be performed to investigate the modes of transmission of *T. cruzi* among wild and peridomiciliary reservoirs and by the invertebrate vector *M. spinolai*.

Received January 17, 2005. Accepted for publication April 6, 2005.

Financial support: This study was supported by grants FONDECYT 104-0762 and IAEA CHI-11762 to A.S. Additional support was obtained from the following grants: FONDECYT 2010022 and 3050033 (C.B.M.) and 1040711 (P.E.C.).

Authors' addresses: Marlene Rozas, Ximena Coronado, Sylvia Ortiz, and Aldo Solari, Program of Cellular and Molecular Biology, Faculty of Medicine, University of Chile, Casilla 70086, Santiago 7, Chile, Telephone: 56-2-6786062, Fax: 56-2-735-5580, E-mail:

asolari@machi.med.uchile.cl. Carezza Botto-Mahan, Department of Ecological Sciences, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile, Telephone: 56-2-6787232, Fax: 56-2-272-7363, E-mail: cbotto@uchile.cl. Pedro E. Cattan, Department of Biological Sciences, Faculty of Veterinary Sciences, University of Chile, Casilla 2 correo 15, Santiago, Chile, Telephone: 56-2-6785629, Fax: 56-2-6785526, E-mail: pcattan@uchile.cl.

REFERENCES

1. OPS/OMS, 2003. XII[a] Reunión de la Comisión Intergubernamental del Cono Sur para la eliminación de *Triatoma infestans* y la interrupción de la transmisión transfusional de la Tripanosomiasis Americana (INCOSUR/Chagas). Santiago, Chile: World Health Organization.
2. Lorca M, Garcia A, Contreras M, Schenone H, Rojas A, 2001. Evaluation of a *Triatoma infestans* elimination program by the decrease of *Trypanosoma cruzi* infection frequency in children younger than 10 years, Chile, 1991–1998. *Am J Trop Med Hyg 65:* 861–864.
3. Lent H, Wygodzinsky P, 1979. Revision of the triatominae (Reduviidae Hemiptera), and their significance as vector of Chagas's disease. *Bull Am Mus Nat Hist 163:* 123–520.
4. Canals M, Cruzat L, Molina MC, Ferreira A, Cattan PE, 2001. Blood host sources of *Mepraia spinolai* (Heteroptera: Reduviidae), wild vector of Chagas disease in Chile. *J Med Entomol 38:* 303–307.
5. Canals M, Ehrenfeld M, Cattan PE, 2000. Situación de *Mepraia spinolai* vector silvestre de la enfermedad de Chagas en Chile, en relación con otros vectores desde la perspectiva de sus fuentes de alimentación. *Rev Med Chile 128*: 1108–1112.
6. Barreto M, 1964. Reservatórios do *Trypanosoma cruzi* nas Américas. *Rev Brasil Malariol D Trop 16:* 27.
7. Telford S, Tonn R, 1982. Dynamics of *Trypanosoma cruzi* in populations of a primary reservoir, *Didelphis marsupialis* in the highland of Venezuela. *Bol Ofic Sanit Panam 93:* 341.
8. Schenone H, Contreras MC, Borgono JM, Maturana R, Salinas P, Sandoval L, Rojas A, Tello P, Villarroel F, 1991. Panorama general de la epidemiología de la enfermedad de Chagas en Chile. *Bol Chil Parasitol 46:* 19–30.
9. Ulloa M, Traslaviña M, Alcaino H, 1989. Enfermedad de Chagas en caninos y caprinos sinantrópicos de la Provincia del Choapa, IV Región, Chile. *Parasitol al día 13:* 120–124.
10. Whiting C, 1946. Contribución al estudio de las reservas de parásitos de la enfermedad de Chagas en Chile. Primeros hallazgos en Chile de mamíferos silvestres infestados por *Trypanosoma cruzi. Rev Chil Hig Med Prev 8:* 69–102.
11. Duran J, Videla M, Apt W, 1989. Enfermedad de Chagas en una comunidad de pequeños mamíferos simpátricos de la Reserva Nacional de Las Chinchillas. *Parasitol al Día 13:* 15–20.
12. Jimenez J, Lorca M, 1990. Trypanosomiasis americana en vertebrados silvestres y su relación con el vector *Triatoma spinolai. Arch Med Vet XXII:* 179–183.
13. WHO, 1991. *Control of Chagas Disease*. WHO Technical Report Series 811. Geneva: World Health Organization, 97.
14. Wincker P, Britto C, Borges J, Cardoso M, Oelemann W, Morel C, 1994. Use a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. *Am J Trop Med Hyg 51:* 771–777.
15. Solari A, Campillay R, Ortiz S, Wallace A, 2001. Identification of *Trypanosoma cruzi* genotypes circulating in Chilean Chagasic patients. *Exp Parasitol 97:* 226–233.
16. Dias-Lima A, Sherlock I, 2000. Sylvatic vectors invading houses and the risk of emergence of cases of Chagas disease in Salvador, State of Bahia, Northeast Brazil. *Mem Inst Oswaldo Cruz 95:* 611–613.