American Journal of Primatology 75:161–169 (2013)

RESEARCH ARTICLE

Seroprevalence of *Trypanosoma cruzi* and *Leishmania mexicana* in Free-Ranging Howler Monkeys in Southeastern Mexico

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Natural infection of wild mammals by protozoa parasites is quite common in nature. For Neotropical Primates different infections of parasites that are etiological agent of disease in human have been identified. In particular, infections by *Trypanosoma cruzi* and *Leishmania* sp., have been reported for some New World primate species, but there are no reports of infection with these parasites in any primate species in Mexico. A serological study was conducted on two howler monkey species (*Alouatta pigra* and *A. palliata*) from the Mexican states of Campeche and Tabasco. A total of 55 serum samples (20 samples from *A. pigra*, 20 samples from *A. palliata*, and 15 samples from semifree ranging *A. palliata* of Los Tuxtlas, Veracruz as negative controls) were analyzed for the detection of immunoglobulin G antibodies against *T. cruzi* and *Leishmania mexicana* through enzyme linked immunosorbent assay test, indirect immunofluorescence assay and Western blot. The overall prevalence of antibodies in howler monkeys was 17.5% for *T. cruzi* and 30% for *L. mexicana*. Our results also indicate that *A. pigra* is more susceptible to develop leishmaniasis than *A. palliata*. Finally, the finding of positive serology in these primates should be given serious consideration for public health, given the potential role of these primate species as wild reservoirs for these diseases and the increasing contact of monkeys with human populations due to habitat loss and fragmentation. Am. J. Primatol. 75:161–169, 2013.

Key words: *Trypanosoma cruzi*; *Leishmania mexicana*; howler monkeys; free-ranging; seroprevalence

INTRODUCTION

The Trypanosomatidae, infecting humans in the Americas belongs to the genera *Leishmania* and *Trypanosoma* [WHO, 1991]. These parasites are endemic in most tropical regions of the world and their life cycles involve obligatory passage through vertebrate and invertebrate hosts comprising several stages [WHO, 2002].

Leishmania are protozoan parasites, transmitted to a susceptible host by phlebotomine sand flies (Diptera: Psychodidae) of the genera *Phlebotomus* causing leishmaniases. This is a group of diseases with diverse epidemiological and clinical patterns from self-healing skin ulcers to a severe, life-threatening visceral disease [Dantas-Torres, 2007]. For example, *Leishmania mexicana* is the parasite responsible for chiclero's ulcer [Grimaldi et al., 1987]. Natural vertebrate hosts of *Leishmania* parasites are mammals of the orders: Edentata, Carnivora, Hyracoidea, Rodentia, Marsupialia, Perissodactyla, and Primates [Ashford, 1996; Gramiccia & Gradoni, 2005; Saliba & Oumeish, 1999].

Trypanosoma cruzi known as Chagas disease is transmitted by the infected feces of blood-sucking triatomine bugs; a hemoflagellate protozoan (Family Trypanosomatidae, Order Kinetoplastida) [Hoare & Wallace, 1966]. Individuals infected by *T. cruzi* sometimes remain asymptomatic, which characterizes the indeterminate form of the disease [Moncayo,

Contract grant sponsor: FOMIX-CONACyT Contract grant numbers: 2008-C02–108783; Contract grant sponsor: National Science Foundation (NSF); Contract grant number: BCS 0962807.

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Received 22 June 2012; revised 06 October 2012; revision accepted 13 October 2012

DOI 10.1002/ajp.22094
Published online 19 November 2012 in Wiley Online Library (wileyonlinelibrary.com).

2003]. However infected individuals may develop irreversible lesions of the autonomous nervous system in the heart, esophagus, and colon, in the chronic phase of the infection [Moncayo, 2003; Prata, 2001]. In the natural environment, hosts of *T. cruzi* include all-mammalian orders, but Didelphimorpha, Xenarthra, Rodentia, and Primate are the most frequently infected [Coura et al., 2002; Miles et al., 1979, 2003].

It is estimated that 69,000 people are infected with *T. cruzi* each year in Mexico [Petherick, 2010] while cases of Leishmaniasis have been reported in 16 of the 31 states of Mexico [Rebollar-Téllez et al., 1996; Rodríguez, 2002].

Mexico has enormous climatic and biological diversity, providing opportunities for the development of etiological agents such as *T. cruzi* and *L. mexicana*. The invasion of human populations into terrestrial ecosystems has resulted in the establishment and spread of these diseases [Cruz-Reyes & Pickering-López, 2006]. Moreover, the great diversity of habitats and poor sanitary conditions in many rural regions, populated by persons of low socioeconomic status, provides natural conditions for transmission resulting in an increase the risk of infection.

Studies suggest that some neotropical primates are reservoirs of microorganisms pathogenic to man [Stuart et al., 1998; Ziccardi & Lourenco-de-Oliveira, 1997]. Neotropical primates are largely arboreal and a number of human activities directly impact their habitat. In many areas across their distribution, monkeys survive in highly disturbed forest fragments in close proximity to human settlements, which increases the risk of disease transmission [Chapman et al., 2005; Daszak et al., 2001; Dobson & Foufopoulos, 2001; Kowalewski & Gillespie, 2009]. This scenario is particularly true for howler monkey in Mexico [Arroyo-Rodríguez & Duarte-Dias, 2009].

Howler monkeys (genus *Alouatta*), have the broadest geographic distribution of New World primates, extending from northern Argentina to southern Mexico. They inhabit a wide variety of environments, from closed-canopy wet evergreen forests, including flooded swamp forests, to open, highly seasonal deciduous and semideciduous woodlands, and gallery forests [Crockett, 1998; Zunino et al., 2001].

In Mexico, there are two species of howler monkeys, *A. pigra* distributed in the states of Tabasco, Campeche, Chiapas, Yucatán, and Quintana Roo [Barrueta-Rath et al., 2003; Estrada et al., 2002] and *A. palliata* distributed in the states of southern Veracruz, Tabasco, and Chiapas [Horwich & Johnson, 1986] and east of the Yucatan Peninsula [García-Orduá, 1995]; both species can be found in highly fragmented habitats. It is known that howler monkeys host various pathogens that also infect livestock and humans [Stuart et al., 1998]. This shared susceptibility to infection holds the possibility of cross-species transmission in disturbed forest systems where howler monkeys experience particularly high temporal and spatial overlap with livestock and humans [Kowalewski & Gillespie, 2009]. *Alouatta* groups can be found living next to (and within) plantations (e.g. cacao, Muñoz et al. [2006]), pastureland, and even within small human settlements [Estrada et al., 2006; García-Orduña & Cortes-Ortíz, pers. obs.] or areas highly disturbed with very small fragments [Bicca-Marques & Calegaro-Marques, 1995]; in those environments, they may even travel on the ground, and drink water from rivers and lagoons [Bravo & Sallenave, 2003; Pozo-Montuy & Serio-Silva, 2007]. Up till now, studies have shown that howler monkeys are hosts to zoonotic gastrointestinal parasites, which produce economic, and health problems, such as *Giardia* sp. [Kowalewski et al., 2011] and *Cryptosporidium* sp. [Santa Cruz et al., 2003]. The objectives of the present study are (1) to determine the seroprevalence for *T. cruzi* and *L. mexicana* in two species of howler monkeys from Mexico, probable reservoirs of these parasites, and (2) to analyze using, Enzyme linked immunosorbent (ELISA), Western blotting, and Indirect immunofluorescence (IIF) assays, whether there are any differences in the infection of both trypanosome spp. between the two species of nonhuman primates.

METHODS

Study Area

Tabasco state has a warm humid climate with an annual rainfall of 1,380 mm. The average annual temperature is 24.1°C, with maximum and minimum averages of 32.1 and 15.9°C, respectively. The collecting localities in Tabasco have a total annual rainfall of 4,014 mm and a mean annual temperature of 25.6 °C, with an average maximum and minimum of 29.2 and 22°C. Campeche has a warm humid climate, with an annual rainfall of 1,600 mm and an average annual temperature is 26°C, with an average maximum and minimum of 29.2 and 22.6°C, respectively.

Animal Capturing and Handling

Fourteen groups of howler monkeys were captured between May 31 and June 11, 2010 in southern Mexico. Eleven of these groups were captured in remnants of forest in Tabasco, and the other three groups were captured in a relatively large forest fragment in Campeche (Fig. 1; Table I). Animals were immobilized with remote drug delivery equipment powered with CO_2 (Telinject®, Agua Dulce, CA), using ketamine hydrochloride (Inoketam® 1000 Virbac, S.A. Lab. Guadalajara, Jal. México; 10–15 mg/kg). Anesthetized individuals were either caught with a net or recovered by hand from the trees, in those cases where the monkey remained attached

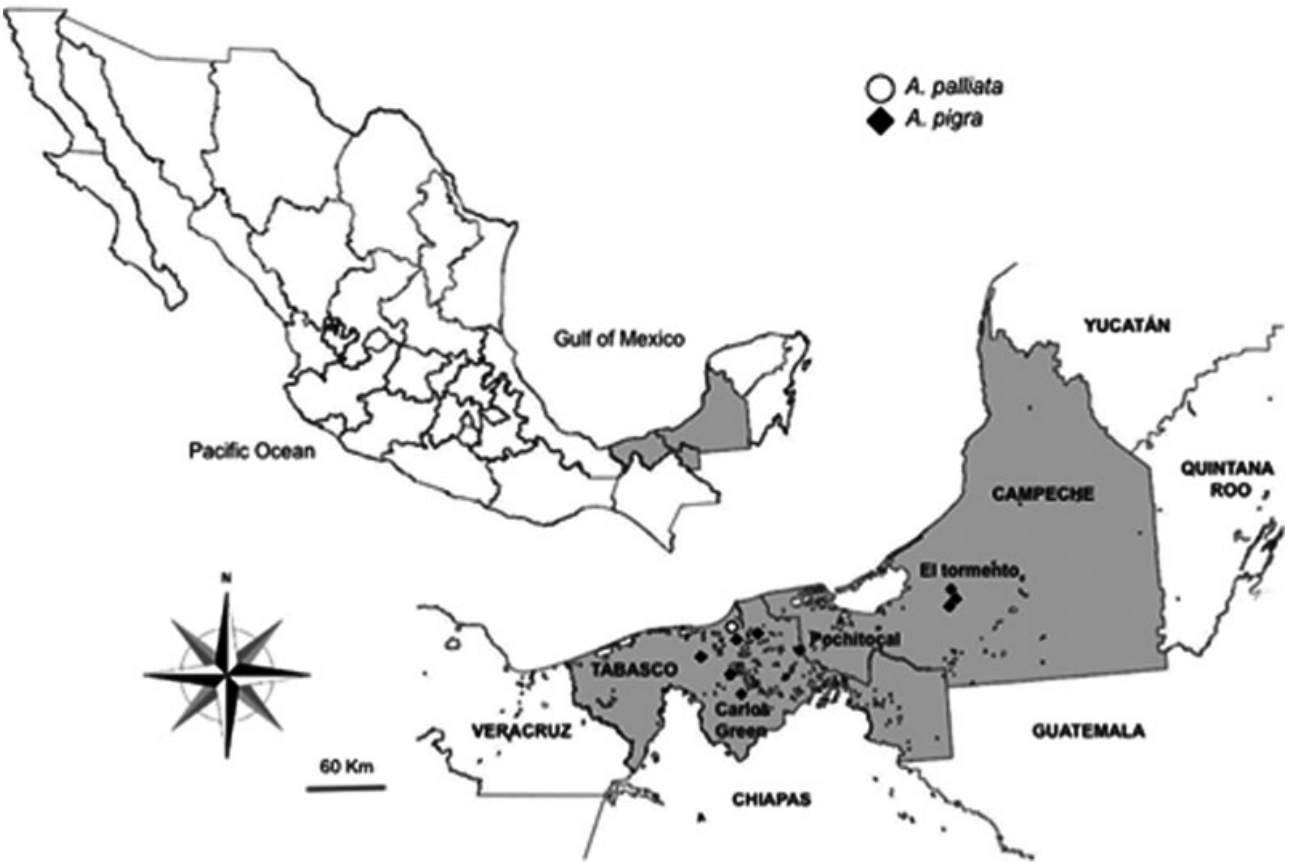

Fig. 1. Map of study area and locations of sampled howler monkeys. Upper left, map shows Mexico with the states of Tabasco and Campeche in grey, and the inlaid map shows the study area in these two states as well as the neighboring states of Veracruz and Chiapas in Mexico, and Guatemala. Grey areas correspond to the states of Tabasco and Campeche, with lines delineating the respective states, including Veracruz, Chiapas, and the neighboring country of Guatemala, with bodies of water in the area. Black dots indicate sampling locations of *A. pigra* and white dots sampling location of *A. palliata*.

to branch by its tail. Individuals were immediately transferred to the staging area where experienced professionals carried out a thorough check-up of the monkeys, and blood samples were collected. After sampling, each individual was placed temporarily in an individual cage until complete recovery from the anesthesia and then were released in the same site where they were captured, to rejoin their social group.

This study was approved by the institutional guidelines of the Universidad Veracruzana, the Mexican Federal Government Agency Ministry of the

TABLE I. IgG Antibodies against to *T. cruzi* and *L. mexicana* in Howler Monkeys

Capture zone		Species			Serology					
					Positive		Positive			
Tabasco	Campeche			Total positive	*T. cruzi*		*L. mexicana*		Double positive	
($n = 28$)	($n = 12$)	*A. palliata*	*A. pigra*	primates	*A. palliata*	*A. pigra*	*A. palliata*	*A. pigra*	*A. palliata*	*A. pigra*
Carlos Green ($n = 3$)		1	2	1/28 (3.6%)	0/1	1/2	0/1	1/2	0/1	1/2
Pochitocal ($n = 25$)		19	6	6/28 (21.4%)	2/19	2/6	1/19	4/6	1/19	2/6
	Tormento ($n = 12$)	0	12	7/12 (58.3%)	-	2/12	-	6/12	- -	1/12
Total		20/40	20/40	14/40 (35%)	2/40 (5%)	7/40 (17.5%)	1/40 (2.5%)	11/40 (27.5%)	1/40 (2.5%)	4/40 (10%)

Environment, and Natural Resources (SEMARNAT; official permits # SGPA/DGVS/03293/10) and was performed according to the American Society of Primatologists' principles for the Ethical Treatment of Non-human Primates.

Sample Collection

A syringe (BD-Vacutainer® 21G × 32 mm, México, DF) was used to collect 2.5 ml of blood from the ventromedial artery of the tail. The serum was separated by centrifugation at 1,200 × *g* for 10 min and samples were immediately refrigerated and transported to the laboratory, where they were stored at –70°C until tested. In total 40 samples (20 *A. palliata* and 20 *A. pigra*) were obtained for this study.

Parasites

Epimastigotes forms of the *T. cruzi* MHOM/MX/1994/INC-1 strain, and the G8 strain of *L. mexicana* were used in this study. The parasites were grown in liver-infusion tryptone broth-LIT supplemented with 10% fetal bovine serum (GIBCO Life Technologies, Corporation) at 28°C [Camargo, 1964], and the G8 strain of *L. mexicana* was cultured in a DMEM medium supplemented with 10% fetal calf serum. These extracts were used in western blotting analysis and indirect IIF assay.

Enzyme Linked Immunosorbent Assay (ELISA)

We used *T. cruzi* and *L. mexicana* crude extracts for ELISA tests [Ramos-Ligonio et al., 2006]. In brief, logarithmic phase parasites were harvested by centrifugation at 1,000 × *g* for 10 min at 4°C; the parasite pellet was suspended in 500 μl Phosphate Buffer Saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM KH_2PO_4, pH 7.4) and lysed by cycles of freezing (–70°C) and thawing (25°C). The lysate was centrifuged at 10,000 × *g* for 20 min at 4°C. The resulting supernatant was used as crude antigen extract. Polystyrene plates (Costar Corporation, Cambridge, MA) were coated with the *T. cruzi* or *L. mexicana* crude antigen extract (2 μg/ml) separately in carbonate buffer, pH 9.6, and incubated overnight at 4°C. The unbound antigen was discarded and the plates were blocked with 200 μl of PBS containing 5% nonfat milk for 2 hr at 37°C. The plates were incubated with 50 μl of serum samples (1:100 dilution), and each plate also included positive and negative control sera. Further washing steps were conducted and a peroxidase labeled goat–antimonkey IgG antibody (Bethyl Laboratories, Inc., Montgomery, TX) was added at a 1:5,000 dilution in PBS/0.05% Tween-20 and incubated for 1 hr at room temperature (RT). After eight washings, 100 μl ABTS (2,2,-azino-bis (3-ethylbenzthiazoline)-6-sulphonic acid; Zymed Life Technologies Corporation) were added as substrate and the reaction was allowed to proceed for 20 min at RT. The reaction was stopped with 2% sulfuric acid, and absorbance was read at 415 nm with an ELISA microplate reader (Multiscan MS, Labsystem Inc., Franklin, MA).

The cut-off for this assay (at dilution 1:100) was established using the average obtained from a sample of 25 apparently healthy monkey sera plus two standard deviations (SD). Positive samples were defined as samples with absorbance greater than two SD above the mean of the negative control.

Western Blotting Analysis (WB)

WB was carried out as described previously [Ramos-Ligonio et al., 2006]. Briefly, crude antigen extracts of *T. cruzi* from the MHOM/MX/1994/INC-1 strain or G8 strain of *L. mexicana* were separated by electrophoresis in a 10% SDS polyacrylamide gel and electroblotted onto a nitrocellulose membrane (Bio-Rad Laboratories, Inc.) at 80 volts at 4°C for 1 hr. The membrane was blocked with a 5% (m/v) solution of nonfat milk powder, washed with Tris-Buffered Saline Tween 20 (TBST) (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05% Tween 20) buffer, and the nitrocellulose membrane was cut into strips that were individually incubated (2 hr at 37 °C) with 1 ml of monkey serum diluted 1:100 in TBST/2% skim milk. The control strips were incubated with positive and negative sera. Each strip was washed three times with TBST and subsequently incubated with phosphatase-alkaline labeled goat–antimonkey IgG (Bethyl Laboratories, Inc., Montgomery, TX). The strips were then washed as above and the immune complexes were developed with NBT (Nitro-blue-tetrazolium; Sigma-Aldrich, Co.) and BCIP (5-bromo-4-chloro-3′-indolylphospahate; Sigma-Aldrich, Co.). The reaction was stopped with water. Positive, negative, and secondary antibody controls were included in each experiment.

IIF Assay

Trypanosoma cruzi epimastigotes from the MHOM/MX/1994/INC-1 strain or *L. mexicana* promastigotes from the G8 strain were washed three times in ice-cold PBS, and 10 μl of a 10^7 cell/ml suspension was allowed to settle in 10-well IIF slides (BioMerieux, Marcy I'Etoile, France) for 30 min at RT in a humidified atmosphere. Cells were fixed by incubation at 60°C and either stored at 20°C (up to 1 month) or used immediately. Slides were blocked with a goat serum (dilution 1:50 in PBS). Parasites were incubated with control or tested sera (monkey's sera) diluted 1:50 in PBS for 30 min a 37°C in a humidified atmosphere and then washed twice in PBS

and once in distilled water. Antibody fixation was revealed with fluorescein isothiocyanate (FITC) conjugated antimonkey IgG (Bethyl Laboratories, Inc., Montgomery, TX) prepared at 1:100 in 0.01% Evans blue for counter staining. The fluorescence intensity was judged microscopically by the brightness, which corresponded to a semiquantitative scale from negative to quadruple-cross positive. Antibody titers were defined as the highest serum dilution that showed a specific fluorescence corresponding to double-cross positive.

Positive controls were pooled sera from patients with Chagas disease and leishmaniasis. Negative controls consisted of sera from semi-free-ranging howler monkeys (15 individuals) of Isla Agaltepec located within Laguna Catemaco Lake in Los Tuxtlas region Veracruz, México, where there are no reported cases of the presence of both diseases. These controls were used in all serological tests.

Data Analysis

A chi-square analysis was used to assess significant differences in seroprevalence rates between species/age classes. All proportion data are presented with their 95% confidence intervals. The proportion data were compared through Fisher's exact tests, and the Kappa index was calculated when applicable.

RESULTS

Detection of Antibodies against to *T. cruzi* and *L. mexicana* through ELISA

Thirty percent (12/40) of the samples came from wild individuals caught in the Nature Reserve "El Tormento" in Campeche, and 70% (28/40) in patches of forest nearby the communities of "Pochitocal" and "Carlos Green" in Tabasco. Seventy-five percent of the samples were from females.

Samples were divided into four groups according to the age class of the individual; 67.5% ($n = 27$) adults, 17.5% ($n = 7$) subadults, 12.5% ($n = 6$) immature (juveniles and infant). All 40 samples were tested for antibodies against *T. cruzi* and *L. mexicana*. Seven samples were positive for anti-*T. cruzi* antibodies, with ELISA assay corresponding to an overall seroprevalence of 17.5% with absorbance values above 0.28 (±2 SD, cut-off point), while 12 samples were positive for antibodies against *L. mexicana*, obtaining a seroprevalence of 30% with absorbance values above 0.30 (±2 SD, cut-off point; Table I). These absorbance values at 415 nm were higher than 2 SD of the mean absorbance values ($n = 25$) of the negative controls (0.16 for *T. cruzi* and 0.20 for *L. mexicana*). The mean absorbance values for test sera scored as positive were 0.885 for *T. cruzi* and 0.987 for *L. mexicana*. In total, 35% of the samples had positive serology; of these, six samples were from "Pochitocal" (Tabasco), one sample from "Carlos Green" (Tabasco), and seven from Campeche, with 21.4%, 3.6%, and 58.3% seroprevalence, in each locality, respectively. Remarkably, 12.5% of all samples (four from Tabasco and one from Campeche) had positive serology for both *T. cruzi* and *L. mexicana* (Table I). Most seropositive individuals were adults, but there was no association between age class and the presence of reactivity to pathogens ($\chi^2 = 1.73$, $df = 2$, $P = 0.42$). A higher proportion of seropositive samples, particularly for *L. mexicana,* were found in Campeche (Fig. 2; Table I).

Of the seven samples that tested positive for anti-*T. cruzi* antibodies by ELISA, six samples were also positive when using the indirect IIF assay (concordance between ELISA and IIF, $\kappa = 0.90 \pm 0.09$), but were not detected using WB test (Table II).

In the case of *L. mexicana*, of the 12 samples that tested positive for anti-*L. mexicana* antibodies using ELISA, 11 samples were also positive with IIF, and nine were positive using WB. The congruence of *L. mexicana* detection with ELISA and IIF was excellent ($\kappa = 0.939 \pm 0.06$), and the congruence between ELISA and WB was also good ($\kappa = 0.808 \pm 1.07$; Table II).

In general, there was a significant association between seropositivity and the species of howler monkey tested ($\chi^2 = 8.90$, d.f = 1, $P = 0.0028$) with *A. pigra* being the most susceptible. This difference was mainly due to the high susceptibility of *A. pigra* to *Leishmania* infections, because no significant association was found between the seropositivity for *T. cruzi* and the primates ($P = 0.407$, Fisher's exact test); but when analyzing the seropositivity for *L. mexicana*, we found a significant difference between species (92% *A. pigra* vs. 8% *A. palliata*, $P = 0.0012$, Fisher's exact test).

DISCUSSION

The purpose of this study was to evaluate the presence of immunoglobulin G antibodies against *T. cruzi* and *L. mexicana* in Mexican howler monkeys and thereby determine if these monkey populations are seropositive to these parasitic diseases. Serum samples of howler monkeys were analyzed by three diagnostic tests (ELISA, IIF, and WB) with total extract of *T. cruzi* and *L. mexicana* parasites.

Thirty-five percent of our samples tested positive for at least one parasite species. The concordance between ELISA and IIF tests was very high for both parasites, which ensured high reliability of the results. However, WB was not sensitive to a proportion of positive cases found with the other tests. This was particularly true for the detection of *T. cruzi*. The low performance of the Western blot test had been observed in previous studies in humans, and

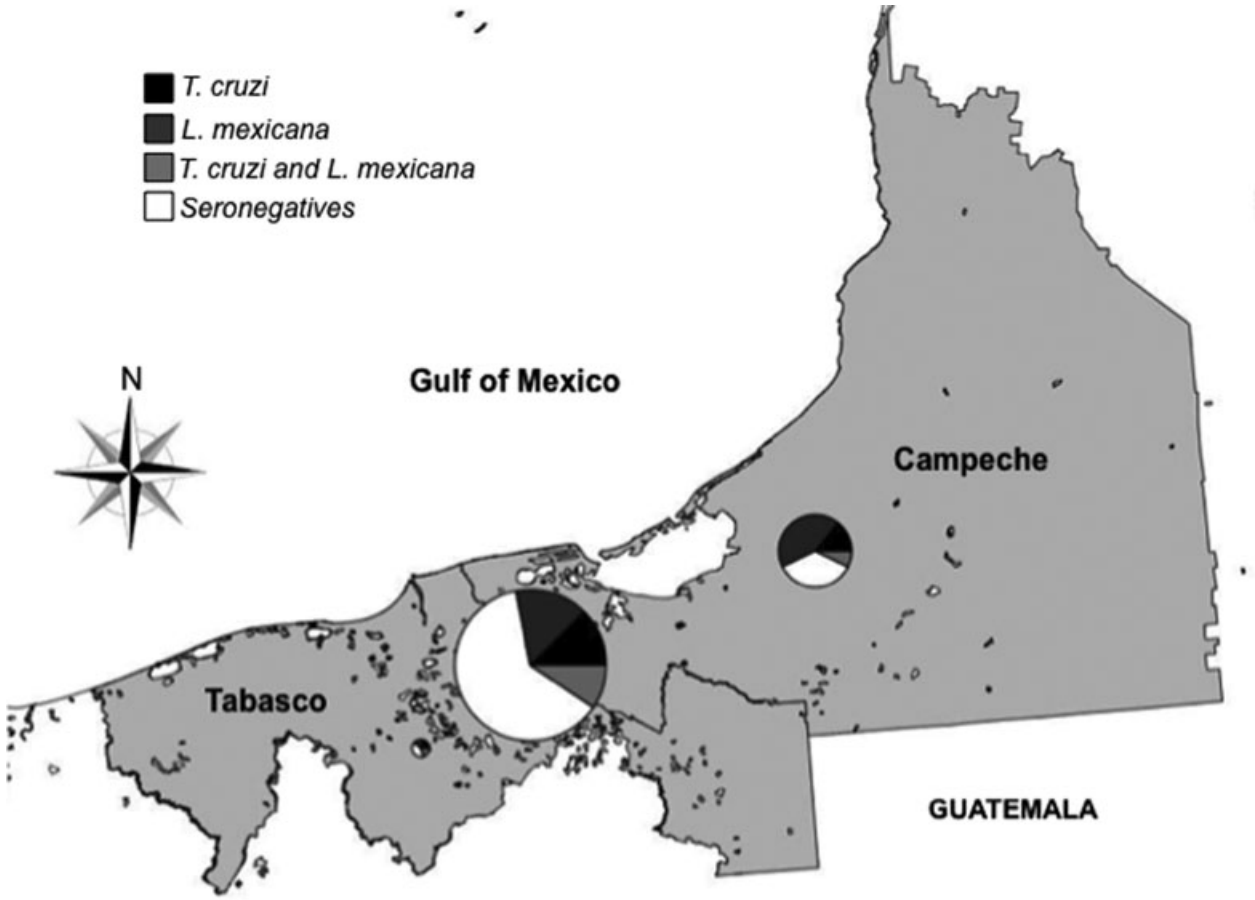

Fig. 2. Geographic distribution of *T. cruzi* and *L. mexicana* seroprevalence in howler monkeys in southeastern Mexico. Circles are proportional to the number of serum samples analyzed in each zone. Black areas in pie charts represent the proportion of *T. cruzi* seropositive monkeys, dark grey areas represent the proportion of *L. mexicana* seropositive monkeys, light grey areas represent the proportion of monkeys seropositive to both parasites, and white areas represent the proportion of seronegatives monkeys in each sampled area.

may be caused by poor recognition of denatured antigens, which suggests that WB may not be an appropriate method for the confirmation of *T. cruzi* infection [Ramos-Ligonio et al., 2010, 2006; Rangel-Flores et al., 2001].

The results of both howler monkey species indicate that *A. pigra* presents a greater percentage of infection for both diseases (Chagas and Leishmania), regardless of the area where they were sampled. This suggests that *A. pigra* is probably more susceptible to parasitic infectious diseases that *A. palliata*.

Trypanosoma cruzi

It is well known that mammals are reservoirs of the etiological agent of many diseases and possess an important role in the maintenance and interaction of domestic, peridomestic, and wild cycles of Chagas disease [Barreto, 1964; Dantas-Torres, 2007; Telford & Tonn, 1982]. In the infectious cycle, the parasite can infect any mammal and the fact that chronically infected animals have persistent parasitaemia, results in an enormous sylvatic, and domestic reservoirs in enzootic regions; this in turn drives the

TABLE II. Summary of Serological Test Results

Tests	ELISA	IIF	WB	Two tests	Three tests
T. cruzi positives/total tested	7/40	6/40	0/40	6/40 $\kappa = 0.90 \pm 0.09$[a]	——
L. mexicana positives/total tested	12/40	11/40	9/40 $\kappa = 0.808 \pm 1.07$[b]	11/40 $\kappa = 0.939 \pm 0.06$[c]	9/40

WB: western blot. IIF: Indirect Immunofluorescence.
[a]ELISA versus IIF, [b]ELISA versus WB, [c]ELISA versus IIF.

establishment of the domiciliary cycle of transmission of the parasite in human dwellings [Kirchhoff, 2011].

There are reports of primate infection by some species of trypanosomes such as *T. minasense*, but most are not of health importance to humans [Chinchilla et al., 2005]. However, infection of *T. cruzi*, the etiological agent of Chagas' disease in humans, has been reported in several species of Neotropical primates [Lisboa et al., 2006; Monteiro et al., 2006], including a number of South American species of howler monkeys [*A. caraya*, Funayama & Baretto, 1970; Travi et al., 1986; *A. seniculus*, de Thoisy, et al., 2001]. However, the prevalence of *T. cruzi* infection in Mexican howler monkeys (*A. palliata* and *A. pigra*) had not been previously been explored. Our present finding of positive serology for *T. cruzi* strongly suggests that these howler monkeys can be naturally infected by the parasite and will develop an asymptomatic disease, and that they might serve as a reservoir [Lisboa et al., 2006; Marinkelle, 1982]. Although Neotropical primates seem to be commonly infected with *T. cruzi*, this infection does not necessarily have a significant impact on the health status of the individuals [de Thoisy et al., 2001]. Infections in species of Old World primates have been detected in captivity [e.g. Gleiser et al., 1986; Zabalgoltia et al., 2003] and one report showed a fatal case of *T. cruzi* in a chimpanzee during the acute phase of the disease [Bommineni et al., 2009]. The majority of the emerging infectious diseases are zoonotic and easily transferred among humans, wildlife, and domestic animals [Nunn & Altizer, 2006]. The probability of human infection by *T. cruzi* increases with the growing contact between humans and no-human primates, due to the loss and fragmentation of forest areas in the Neotropics [Kowalewski & Gillespie, 2009].

Leishmania mexicana

The consistency of detection of *L. mexicana* infection using three tests indicates the high reliability of our results. *Leishmania* infection has been previously documented in Old World [Gicheru et al., 2009] and New World [Lainson et al., 1988] primates. However, as far as we are aware, there is only one previous report of a natural infection with *Leishmania* in howler monkeys (*Alouatta guariba*), although their species was not identified [Malta et al., 2010]. *Leishmania* sp. can naturally infect a wide range of commensal mammalian species such as cats [McCown & Grzeszak, 2010; Vides et al., 2011] dogs [Freeman, 2010] and rodents [Akhavan et al., 2010], several wildlife species such as wolves and marsupials, and many others from several orders, as already mentioned above. These species can serve as reservoirs of the parasite in a wild environment. Nonetheless, these wildlife host species are usually not believed to be responsible for transmission to humans and their epidemiological significance as reservoirs is still debatable [Diniz et al., 2008; Luppi et al., 2008]. On the contrary, the present serological results regarding their reactivity against proteins of *L. mexicana* and an apparent absence of symptoms strongly suggest that these primates could play an important role in the transmission process as a potential natural reservoir for the parasite.

Implications for Conservation and Public Health

The fact that the parasites examined in this study are transmitted primarily through blood (transmitted by insects), suggest that if howler monkeys become infected by these parasites, these parasitic diseases could potentially be transmitted to humans and to domestic animals. Then in turn they might serve as reservoirs and a nutritional supply for the vector, which is reported to be present in the area under study for these diseases [Canto-Lara et al., 1998, González et al., 2011]. Many parasitic species and their potential hosts have coexisted for a long time [Nunn & Altizer, 2006], but it is not clear what consequences, if any, constant exposure to parasites may have on the survival of wild populations of primates [Gillespie, 2006; Gillespie et al., 2004]. The increased alteration of wild primate habitats is not only affecting the conservation of the primates, but also potentiates the likelihood of disease transmission between non-human primates and humans [Lilly et al., 2002; Wolfe et al., 1998]. The finding in howler monkeys of positive serology for *T. cruzi* and *L. mexicana*, two protozoan parasites that cause infections of importance to public health, deserve special attention. More studies on the parasitic strains present in these monkeys, as well as on the role that these primate species play in the life cycle of these parasites are needed to provide more information about the host–parasite–vector relationship and to provide a better understanding of the dynamics of the transmission of these diseases in Southern Mexico.

ACKNOWLEDGMENTS

The authors would like to thank the team of the University of Veracruz who assisted in the capturing and sampling of primates, particularly Javier Hermida, Antonio Jauregui, Antonio Jauregui Jr., Socorro Aguilar Cucurachi, Ariadna Rangel, and Pedro Dias. The study was funded by FOMIX-CONACyT, Veracruz; México (grant 2008-C02–108783) and by the National Science Foundation (NSF) grant no. BCS 0962807. We express special thanks to Warren Haid for his comments to the manuscript.

REFERENCES

Akhavan AA, Mirhendi H, Khamesipour A, Alimohammadian MH, Rassi Y, Bates P, Kamhawi S, Valenzuela JG, Arandian MH, Abdoli H, Jalali-zand N, Jafari R, Shareghi N, Ghanei M, Yaghoobi-Ershadi MR. 2010. *Leishmania* species: detection and identification by nested PCR assay from skin samples of rodent reservoirs. Exp Parasitol 126:552–556.

Arroyo-Rodríguez V, Duarte-Dias PA. 2009. Effects of habitat fragmentation and disturbance on howler monkeys: a review. Am J Primatol 71:1–16

Ashford RW. 1996. Leishmaniasis reservoirs and their significance in control. Clin Dermatol 14:523–532.

Barreto M. 1964. Reservatórios do *Trypanosoma cruzi* nas Américas. Rev Bras Malariol Doencas Trop 16:27–48.

Barrueta-Rath T, Estrada A, Pozo C, Calmé S. 2003. Reconocimiento demográfico de *Alouatta pigra* y *Ateles geoffroyi* en la reserva El Tormento, Campeche, México. Neotrop Primates 11(3):163–167.

Bicca-Marques JC, Calegaro-Marques C. 1995. Locomotion of black howlers in hábitat with discontinuous canopy. Folia Primatol 64:55–61.

Bommineni YR, Dick EJ, Jr., Estep JS, Van de Berg JL, Hubbard GB. 2009. Fatal acute Chagas disease in a chimpanzee. J Med Primatol 38:247–251.

Bravo SP, Sallenave A. 2003. Foraging behavior and activity patterns of *Alouatta caraya* in the northeastern Argentinean flooded forest. Int J Primatol 24:825–846.

Camargo EP. 1964. Growth and differentiation in *Trypanosoma cruzi.* I. Origin of metacyclic trypanosomes in liquid media. Rev Inst Med Trop São Paulo 6:93–100.

Canto-Lara SB, Cardenas-Maruffo MF, Vargas-Gonzalez A, Andrade-Narvaez F. 1998. Isoenzyme characterization of *Leishmania* isolated from human cases with localized cutaneous leishmaniasis from the State of Campeche, Yucatan Peninsula, Mexico. Am J Trop Med Hyg 58:444–447.

Chapman CA Gillespie TR, Goldberg TL. 2005. Primates and the ecology of their infectious diseases: how will antropogenic change affect host-parasite interactions? Evol Antropol 14:134–144.

Chinchilla M, Troyo A, Guerrero OM, Gutierrez-Espeleta GA, Sánchez R. 2005. Presencia de *Trypanosoma minasense* (Kinetoplastida: Trypanosomatidae) en *Alouatta palliata* (Primates: Cebidae) de Costa Rica. Parasitol Latinoam 60:90–92.

Coura JR, Junqueira AC, Fernandez O, Valente SA, Miles MA. 2002. Emerging Chagas disease in Amazonian Brazil. Trends Parasitol 18:171–176.

Crockett CM. 1998. Conservation biology of the genus *Alouatta*. Int J Primatol 19:549–578.

Cruz-Reyes A, Pickering-López JM. 2006. Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years – A Review. Mem Inst Oswaldo Cruz 101(4):345–354.

Dantas-Torres F. 2007. The role of dogs as reservoirs of *Leishmania* parasites, with emphasis on *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Viannia*) *braziliensis*. Vet Parasitol 149:139–146.

Daszak P, Cunningham AA, Hyatt AD. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop 78:103–116.

de Thoisy B, Vogel I, Reynes JM, Pouliquen JF, Carme B, Kazanji M, Vié JC. 2001. Health evaluation of translocated free-ranging primates in French Guiana. Am J Primatol 54:1–16.

Diniz SA, Silva FL, Carvalho Neta AV, Bueno R, Guerra RM-SNC, Abreu-Silva AL, Santos RL. 2008. Animal reservoirs for visceral leishmanisis in densely populated urbana areas. J Infect Dev Ctries 2:24–33.

Dobson A, Foufopoulos J. 2001. Emerging infectious pathogens of wildlife. Philos Trans R Soc Lond B 356:1001–1012.

Estrada A, Castellanos L, Mendoza A, Pacheco R. 2002. Población, ecología y comportamiento de monos aulladores (*Alouatta pigra*) en Palenque, Chiapas, México. In: Lakamha, Boletín Informativo del Museo de sitio y Zona Arqueológica de Palenque (CONACULTA-INAH) 3:9–15.

Estrada A, Saenz J, Harvey C, Naranjo E, Muñoz D, Rosales-Meda M. 2006. Primates in agroecosystems: conservation value of some agricultural practices in Mesoamerican landscapes. In: Estrada A, Garber PA, Pavelka MSM, Luecke L, editors. New perspectives in the study of Mesoamerican primates: distribution, ecology, behavior and conservation. New York: Springer. p 437–470.

Freeman K. 2010. Update on the diagnosis and management of *Leishmania spp* infections in dogs in the United States. Top Companion Anim Med 25:149–154.

Funayama GK, Baretto MP. 1970. Estudos sobre reservatorios e vectores silvestres do *Trypanosoma cruzi*. XLII. Infecco natural do simio *Alouatta caraya* (Humboldt, 1812) pelo *T. Cruzi*. Rev Inst Med Trop São Paulo 12(4):257–265.

García-Orduña F. 1995. Fragmenación del hábitat y demografía de primates en la región de Los Tuxtlas, Ver. In: Rodríguez-Luna E, Cortés-Ortíz L, Martínez-Contreras J, editors. Estudios primatológicos en México. Mexico: Universidad Veracruzana (Ed). vol II. p 61–80.

Gicheru MM, Jeneby MM, Macharia JC, Carlsson HE, Suleman MA. 2009. Prevalence of antibodies and cell mediated immune response against Leishmania major in feral nonhuman primates from Kenya. Acta Trop 109:136–140.

Gillespie TR. 2006. Non-invasive assessment of gastrointestinal parasite infections in free-ranging primates. Int J Primatol 27:1129–1143.

Gillespie TR, Greiner EC, Chapman CA. 2004. Gastrointestinal parasites of the guenons of western Uganda. J Parasitol 90:1356–1360.

Gleiser CA, Yaeger RG, Ghidoni JJ. 1986. Trypanosoma cruzi infection in a colony-born baboon. J Am Vet Med Assoc 189:1225–1226.

González C, Rebollar-Téllez EA, Ibáñez-Bernal S, Becker-Fauser I, Martínez-Meyer E, Peterson AT, Sánchez-Cordero V. 2011. Current knowledge of *Leishmania* vectors in Mexico: how geographic distributions of species relate to transmission areas. Am J Trop Med Hyg 85:839–846.

Gramiccia M, Gradoni L. 2005. The current status of zoonotic leishmaniases and approaches to disease control. Int J Parasitol 35:1169–1180.

Grimaldi G, David JR, McMahon-Pratt D. 1987. Identification and distribution of New World *Leishmania* species characterized by serodeme analysis using monoclonal antibodies. Am J Trop Med Hyg 36:270–287.

Hoare CA, Wallace FG. 1966. Developmental stages of trypanosomatid flagellates: a new terminology. Nature 244:69–70.

Horwich RH, Johnson ED. 1986. Geographical distribution of the black howler (*Alouata pigra*) in Central America. Primates 27(1):53–62.

Kirchhoff LV. 2011. Trypanosomiasis (Chagas disease). In: Weiss LM, Tanowitz HB, Kirchhoff LV, editors. Advances in parasitology: Chagas disease, Part A. Amsterdam: Elsevier Academic Press. vol. 75. p 1–19.

Kowalewski MM, Gillespie TR. 2009. Ecological and anthropogenic influences on patterns of parasitism in free-ranging primates: a meta-analysis of Genus *Alouatta*. In: Garber PA, Estrada A, Bica-Marquez JC, Heymann EW, Strier B, editors. South American primates: comparative perspectives in the study of behavior, ecology and conservation. New York: Springer Press. vol. 17. p 433–461.

Kowalewski MM, Salzer JS, Deutsch JC, Raño M, Kuhlenschmidt MS, Gillespie TR. 2011. Black and Gold howler monkeys (Alouatta caraya) as sentinels of ecosystem health: patterns of zoonotic protozoa infection relative to degree of human–primate contact. Am J Primatol 73:75–83.

Lainson R, Shaw JJ, Braga RR, Ishikawa EAY, de Souza AAA, Silveira FT. 1988. Isolation of Leishmania from monkeys in the Amazon region of Brazil. Trans R Soc Trop Med Hyg 82:231.

Lilly AA, Mehlman PT, Doran D. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika Research site, Dzanga-Ndoki National Park, Central African Republic. Int J Primatol 23:555–573.

Lisboa CV, Mangia RH, Luz SL, Kluczkovski A, Jr., Ferreira LF, Ribeiro CT, Fernandes O, Jansen AM. 2006. Stable infection of primates with *Trypanosoma cruzi* I and II. Parasitology 133:603–611.

Luppi MM, Malta MCC, Silva TMA, Silva FL, Motta ROC, Miranda I, Ecco R, Santos RL. 2008. Visceral leishmaniasis in wild canids in Brazil. Vet Parasitol 155:146–151.

Malta MC, Tinoco HP, Xavier MN, Vieira AL, Costa EA, Santos RL. 2010. Naturally acquired visceral leishmanisis in non-human primates in Brazil. Vet Parasitol 169:193–197.

Marinkelle CJ. 1982. The prevalence of *Trypanosoma (Schizotrypanum) cruzi* infection in Colombian monkeys and marmosets. Ann Trop Med Parasitol 76:121–124.

McCown M, Grzeszak B. 2010. Zoonotic and infectious disease surveillance in Central America: Honduran feral cats positive for *Toxoplasma*, *Trypanosoma*, *Leishmania*, *Rickettsia*, and Lyme disease. J Spec Oper Med 10:41–43.

Miles MA, Feliciangeli MD, de Arias AR. 2003. American trypanosomiasis (Chagas' disease) and the role of molecular epidemiology in guiding control strategies. Br Med J 28:1444–1448.

Miles MA, Marsden PD, Pettitt LE, Draper CC, Watson S, Seah SK, Hutt MS, Fowler JM. 1979. Experimentala *Trypanosoma cruzi* infection in rhesus monkeys 111. Electrocardiographic and histopathological findings. Trans R Soc Trop Med Hyg 73:528–532.

Moncayo A. 2003. Chagas disease: current epidemiological trends after the interruption of vectorial and transfusional transmission in the southern cone countries. Mem Inst Oswaldo Cruz 95:577–591.

Monteiro RV, Baldez J, Dietz J, Baker A, Lisboa CV, Jansen AM. 2006. Clinical, biochemical and electrocardiographic aspects of *Trypanosoma cruzi* infection in free-ranging golden lion tamarins (*Leontopithecus rosalia*). J Med Primatol 35:48–55.

Muñoz D, Estrada A, Naranjo E, Ochoa S. 2006. Foraging ecology of howler monkeys in a cacao (*Theobroma cacao*) plantation in Comalcalco, Mexico. Am J Primatolo 68:127–142.

Nunn CL, Altizer SM. 2006. Infectious diseases in primates: behavior, ecology and evolution. New York: Oxford University Press. p 159–216.

Petherick A. 2010. Country by country. Nature 465:S10–S11.

Pozo-Montuy G, Serio-Silva JC. 2007. Movement and resource use by a group of *Alouatta pigra* in a forest fragment in Balancán, México. Primates 48:102–107.

Prata A. 2001. Clinical and epidemiological aspects of Chagas disease. Lancet Infect Dis 1:92–100.

Ramos-Ligonio A, López-Monteon A, Guzmán-Gómez D, Rosales-Encina JL, Limón-Flores Y, Dumonteil E. 2010. Identification of a hyperendemic area for *Trypanosoma cruzi* infection in central Veracruz, Mexico. Am J Trop Med Hyg 83:164–70.

Ramos-Ligonio A, Ramirez-Sánchez ME, González-Hernández JC, Rosales-Encina JL, López-Monteon A. 2006. Prevalence of antibodies against *Trypanosoma cruzi* in blood bank donors from the IMSS General Hospital in Orizaba, Veracruz, México. Salud Publica Mex 48:13–21.

Rangel-Flores H, Sánchez B, Mendoza-Duarte J, Barnabé C, Brenière C, Ramos C, Espinoza B. 2001. Serologic and parasitologic demonstration of *Trypanosoma cruzi* infections in an urban area of central Mexico: correlation with electrocardiographic alterations. Am J Trop Med Hyg 65:887–895.

Rebollar-Téllez EA, Ramírez-Fraire, Andrade-Narvaez FJ. 1996. A two years study on vectors of cutaneous Leishmaniasis. Evidence for sylvatic transmission cycle in the State of Campeche, Mexico. Mem Inst Oswaldo Cruz 91(5):555–560.

Rodríguez DL. 2002. Enfermedades transmitidas por vector en México. Rev Fac Med Univ Nac Auton Mex 43(3):126–341.

Saliba EK, Oumeish YO. 1999. Reservoir host of cutaneous leishmaniasis. Clin Dermatol 17:257–277.

Santa Cruz ACM, Borda JT, Patiño EM, Prieto OH, Scheibler N, González AO, Comolli JA, Zunino GE, Gómez LG. 2003. Criptosporidiosis en mono aullador (*Alouatta caraya* Humboldt, 1812) en semicautiverio en Corrientes, Argentina. Comunicaciones Científicas y Tecnológicas, Universidad Nacional del Nordeste, Argentina.

Stuart M, Pendergast V, Rumfelt S, Pierberg S, Greenspan L, Glander K, Clarke M. 1998. Parasites of wild howlers (*Alouatta* spp.). Int J Primatol 19:493–512.

Telford S, Tonn R. 1982. Dynamics of *Trypanosoma cruzi* in populations of a primary reservoir, *Didelphis marsupialis* in the highland of Venezuela. Bol Of Sanit Panam 93:341–364.

Travi BL, Colillas OJ, Segura EL. 1986. Natural trypanosome infection in Neotropical monkeys with special reference to *Saimiri sciureus*. In: Taub, DM, King, FA, editors. Current perspectives in primate biology. New York: Van Nostrand Reinhold. p 296–306.

Vides JP, Schwardt TF, Sobrinho LS, Marinho M, Laurenti MD, Biondo AW, Leutenegger C, Marcondes M. 2011. *Leishmania chagasi* infection in cats with dermatologic lesions from an endemic area of visceral leishmaniosis in Brazil. Vet Parasitol 178:22–28.

Wolfe ND, Escalante AA, Karesh WB, Kilbourn A, Spielman A, Lal AA. 1998. Wild primate populations in emerging infectious disease research: the missing link? Emerg Infect Dis 4:149–158.

World Health Organization. 1991. Chagas' disease: progress in research 1989–1990 tropical diseases. Geneva: WHO. p 69–77.

World Health Organization. 2002. Control of Chagas disease (2nd edition). Technical Reports Series 905. Geneva: WHO.

Zabalgoltia M, Ventura J, Anderson L, Carey KD, Williams JT, Vandeberg JL. 2003. Morphologic and functional characterization of chagasic heart disease in non-human primates. Am J Trop Med Hyg 68(2):248–252.

Ziccardi M, Lourenco-de-Oliveira R. 1997. The infection rates of trypanosomes in squirrel monkeys at two sites in the Brazilian Amazon. Mem Inst Oswaldo Cruz 92:465–470.

Zunino GE, González V, Kowaleski MM, Bravo SP. 2001. *Alouatta caraya*. Relationships among habitat, density and social organization. Primate Rep 61:37–46.