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Low Prevalence of Chagas Parasite Infection in a Nonhuman Primate Colony in Louisiana

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Chagas disease, an important cause of heart disease in Latin America, is caused by the parasite *Trypanosoma cruzi*, which typically is transmitted to humans by triatomine insects. Although autochthonous transmission of the Chagas parasite to humans is rare in the United States, triatomines are common, and more than 20 species of mammals are infected with the Chagas parasite in the southern United States. Chagas disease has also been detected in colonies of nonhuman primates (NHP) in Georgia and Texas, and heart abnormalities consistent with Chagas disease have occurred at our NHP center in Louisiana. To determine the level of *T. cruzi* infection, we serologically tested 2157 of the approximately 4200 NHP at the center; 34 of 2157 primates (1.6%) tested positive. Presence of the *T. cruzi* parasite was confirmed by hemoculture in 4 NHP and PCR of the cultured parasites. These results strongly suggest local transmission of *T. cruzi*, because most of the infected NHP were born and raised at this site. All 3 species of NHP tested yielded infected animals, with significantly higher infection prevalence in pig-tailed macaques, suggesting possible exploration of this species as a model organism. The local *T. cruzi* strain isolated during this study would enhance such investigations. The NHP at this center are bred for use in scientific research, and the effects of the Chagas parasite on infected primates could confuse the interpretation of other studies.

Abbreviations: NHP, nonhuman primate; TNPRC, Tulane National Primate Research Center.

Most nonhuman primates (NHP) used in research in the United States are now raised at 1 of 8 National Primate Research Centers or other institutions in the United States and are shipped as needed for studies, thereby avoiding the importation of pathogens from their native countries. However, knowledge about the infection status of these research animals within colonies in the United States is important with regard to colony health, the outcome of the scientific studies that use these NHP, and the safety of caregivers and laboratory workers.

Infection with the hemoflagellate parasitic pathogen *Trypanosoma cruzi*, the causative agent of Chagas disease, has been reported sporadically in NHP colonies in the United States (Table 1). Chagas is the most serious parasitic disease in Latin America, and despite control efforts, its negative socioeconomic impact is rising.[33] Of the approximately 8 million people infected,[26] 20% to 30% will develop chronic disease; most of these persons will die of heart disease (70% to 85%), digestive disorders (15% to 30%), or neurologic disease (less than 5%). Most (80%) transmission occurs through insect vectors, specifically through contact of parasite-containing feces (which are deposited while the insect is taking a blood meal) with mammalian mucous membranes or through a break in the skin. In addition, approximately 1% to 12% of offspring of infected mothers will acquire the parasite by congenital transmission.[7] Insect-vector-mediated autochthonous transmission to humans is rare in the United States, with only 7 documented cases.[11,18] However, a robust sylvan cycle exists in the United States, including *T. cruzi*-infected triatomine insects and a variety of mammals.[31,36]

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T. cruzi was first identified in the United States in 1916 in the triatomine insect vector *Triatoma protracta*.[19] Eleven species of triatomine insects live in the southern two-thirds of the United States, where they are commonly known as 'kissing bugs' (because as night feeders, they often feed on the face) or 'cone-nosed bugs.' *T. sanguisuga* is the species reported most commonly in Louisiana.[10] On average, the prevalence of infection in the insect vectors is 25%,[36] although much higher prevalence is reported in some areas, including Louisiana (56%).[10] *T. cruzi* has been identified in more than 20 mammalian species across the southern United States; the most important of these mammals are rodents, raccoons, opossums, and armadillos.[6,16] Recent studies showed that the highest prevalence of antibodies against *T. cruzi* occurred in raccoons (0% to 68%, range depends on state) and opossums (17% to 52%).[6]

The first case of *T. cruzi* in a NHP in the United States occurred at the Delta Regional Primate Research Center (Covington, LA; now called the Tulane National Primate Research Center [TNPRC]), where a gibbon (*Hylobates pileatus*) from Malaysia died of symptoms of Chagas disease[30]. This case suggested local transmission because Chagas is endemic only to the Americas (Table 1). Additional cases of *T. cruzi* infection in NHP have been reported in Louisiana, Texas, and Georgia. Additional infected NHP were identified in Washington, Oregon, and Maryland; the suspicion was that they previously were infected in Texas, Louisiana, or Georgia (Table 1). Because these NHP were either imported from outside the United States, where *T. cruzi* has not been reported to occur naturally, or were born and raised in the United States, these cases point to *T. cruzi* infection that was acquired in the United States. To understand the prevalence of locally acquired *T. cruzi* infection at the TNRPC, we conducted a serologic survey of 2157 NHP residing at the center.

Table 1. Reports of *T. cruzi* infection in nonhuman primates in the United States

Species	No. of NHP infected/ no. tested	State where infection identified (and likely acquired)	Evidence (method of detection)	Reference
Pileated gibbon *(Hylobates pileatus)*	1/1	Louisiana	Amastigotes in myocardium at necropsy	30
Rhesus macaque *(Macaca mulatta)*	1/1	Maryland (Texas or Georgia)	Blood culture after inadvertently transferred to immunosuppressed NHP; recipient confirmed by blood smears, serology, and xenodiagnosis	9
Rhesus macaque	20/236 (8.5%)	Texas	Index case: amastigotes observed at necropsy; 19 additional by serology	17
Squirrel monkey *(Saimiri sciureus)*	2/2	Louisiana	Microscopy, hemoculture, and xenodiagnosis	12
Yellow baboon *(Papio cynocephalus)*	1/1	Texas	Amastigotes noted at necropsy	13
Crested black macaque *(Macaca nigra)*	1/1	Oregon (Texas)	Flagellates observed in spinal fluid; amastigotes in brain at necropsy; serology	25
Lion-tailed macaque *(Macaca silenus)*	7/11 (64%)	Georgia	Hemoculture and PCR	27
Ring-tailed lemur *(Lemur catta)*	1/19 (5%)	Georgia	Hemoculture and PCR	27
Pig-tailed macaque *(Macaca nemestrina)*	1/1	Washington (Louisiana)	Hemoculture, PCR, and serology	29
Ring-tailed lemur *(Lemur catta)*	21/41 (51%)	Georgia	Hemoculture, PCR, and serology	14
Black-eyed lemur (*Eulemur macaco flavifrons)*	1/5 (20%)	Georgia	Serology	14
Black and white ruffed lemur (*Varecia variegata variegata)*	3/4 (75%)	Georgia	Serology	14
Chimpanzee (*Pan troglodytes)*	1	Texas	Amastigotes on necropsy, PCR, and immunohistochemistry	5

Materials and Methods

Study design. To determine the prevalence of *T. cruzi* infection in NHP at the TNPRC, we first validated an immunochromatographic rapid, dipstick assay for the detection of *T. cruzi* antibodies in NHP. We then used this test to assay plasma samples from 2157 NHP from the center. *T. cruzi* infection in 4 seropositive NHP was confirmed by hemoculture and PCR of the cultured parasites.

Study site. TNPRC is an AAALAC-accredited facility and 1 of 8 national NHP centers funded by the NIH. The center houses approximately 4200 NHP including 13 different species; the majority are rhesus macaques (*Macaca mulatta*). The NHP that compose the breeding colonies are housed in single-species groups in 70 outdoor corrals and field cages, where the animals have the potential to come into contact with triatomine vectors living in the area. The colonies are managed in accordance with all IACUC regulations that prescribe the humane care and use of laboratory animals (Animal Welfare Act[1] and the *Guide for the Care and Use of Laboratory Animals*).[15] The NHP were fed a standard primate diet supplemented with fresh fruits, vegetables, and forage (seed and nut) mix with water available ad libitum.

Validation of immunochromatographic dipstick assay. The immunochromatographic dipstick assay (Trypanosoma Detect Rapid Test, InBios International, Seattle, WA) has been shown to be a sensitive and specific assay for detecting *T. cruzi* antigens in human sera.[21] This assay was tested on 7 archived NHP serum samples that had previously tested positive for *T. cruzi* antibodies by using 1 of 3 serologic tests (enzyme immunosorbance assay, indirect immunofluoresence, and complement fixation) at the Centers for Disease Control and Prevention. In addition, the dipstick assay was tested on 16 NHP samples positive for the nonpathogenic, but antigenically similar, *T. rangeli* parasite and 10 NHP serum samples negative for both parasites (all provided by the Centers for Disease Control and Prevention,

Atlanta, GA). Each dipstick assay on these control sera was performed (in triplicate) according to the manufacturer's instructions. We tested for agreement between the dipstick assay and the archived NHP controls with the κ agreement statistic (κ = 1 is complete agreement; 0 is random) by using JMP version 9 (SAS, Cary, NC).

Study animals. Whole blood was collected from 2172 NHP at the TNPRC by venipuncture into tubes containing EDTA during routine health exams between 2003 to 2004 under a protocol approved by the animal care and use committees of both the TNPRC and Loyola University New Orleans. For sample collection, NHP were anesthetized with an intramuscular injection of ketamine hydrochloride (10 mg/kg); 15 plasma samples were hemolyzed and therefore not analyzed further. Three primate species were tested: 1311 rhesus macaques (*M. mulatta*), 388 pig-tailed macaques (*M. nemestrina*), and 458 baboons (*Papio* spp. [primarily *P. anubis* and *P. cynocephalus* hybrids]). These NHP were residing in 33 of the primate center's 70 outdoor housing areas. The average daily census of the species studied during 2004 (only 31 samples were collected in 2003) was 2776 rhesus macaques, 388 pig-tailed macaques, and 548 baboons. The colony is maintained with more female than male animals as breeding populations; the male-to-female ratio was 1:1.64 for rhesus macaques, 1:3.34 for pig-tailed macaques, and 1:2.81 for baboons.

Assessment of infection. ***Serology.*** The NHP plasma samples were transferred to Loyola University New Orleans, stored at −20 °C, and brought to room temperature before serologic testing. Each of the plasma samples was tested for the presence of antibodies against *T. cruzi* by using the dipstick assay (Trypanosoma Detect Rapid Test, InBios International) according to the manufacturer's instructions. Because of the large number of samples, each experimental sample was tested once, and positive plasma samples were retested in duplicate. All specimens showed identical results on all 3 replicates.

Hemoculture and PCR. To further confirm the presence of *T. cruzi* in serologically positive samples, an additional 5 mL of blood from 7 of the serologically positive NHP underwent hemoculture of the buffy coat according to standard procedures.[27] In addition, PCR using *T. cruzi*-specific primers (the minicircle primers TC3 and TC4) was performed on DNA isolated from parasites cultured from the blood.[11]

Microscopy. The buffy coat from the first approximately 100 blood samples was examined by using wet mounts (magnification, 40×) and Giemsa-stained blood smears (magnification, 100×) for 2 min each by trained observers.

Statistical analysis. We tested for differences in infection prevalence among species by logistic regression. The Fisher exact test was used to test for sex-biased infection prevalence for each species. All statistical analyses were done by using JMP version 9 (SAS). A *P* value less than 0.05 was considered significant.

Results

Validation of the rapid test—control samples. The dipstick assay showed a positive result on 6 of 7 *T. cruzi*-positive control NHP serum samples. Furthermore, all 16 *T. rangeli*-positive control NHP serum samples tested negative for antibodies against *T. cruzi* by the dipstick assay. All 10 negative-control NHP serum samples also were negative by the dipstick assay. The κ coefficient (κ = 0.90, SEM = 0.09, $P < 0.0001$) indicated strong agreement between the dipstick assay and the archived NHP controls. Assays performed in triplicate showed identical results, demonstrating excellent reproducibility.

Serologic testing of the NHP plasma samples. Of the 2157 NHP plasma samples, 34 (1.6%) tested positive for *T. cruzi* antibodies by the dipstick assay. These data included all 3 species: pig-tailed macaques (*M. nemestrina*, 12 [35%] of the 34 positive samples), rhesus macaques (*M. mulatta*, 14 [41%] of the positive samples), and baboons (*Papio* spp., 8 [24%] of the positive samples). The species showing the highest infection prevalence was pig-tailed macaques (3.1% of those tested), followed by baboons (1.7% of those tested) and rhesus macaques (1.1% of those tested). Statistical analysis using logistic regression indicated that infection prevalence was higher in pig-tailed macaques than in rhesus macaques (likelihood ratio: $\chi^2 = 5.13$, $P < 0.05$, Figure 1). The infected primates were dispersed throughout the TNPRC, with positive NHP found in 17 of the 33 corrals and field cages tested.

Microscopy, hemoculture, and PCR. None of the wet mounts or Giemsa-stained blood smears showed trypanosomes, but these methods are known to lack sensitivity in chronically infected mammals.[22]

To confirm that the positive serology accurately reflected *T. cruzi* infection, hemoculture was performed by using blood from 7 of the 34 seropositive NHP. Within 6 wk, trypanosomes appeared in 4 of these cultures (Figure 2). DNA isolated from the cultured trypanosomes was confirmed as *T. cruzi* by PCR using *T. cruzi*-specific primers[11] and obtaining the characteristic 276-bp fragment (data not shown).

Of the 34 NHP that tested positive, most (25 of 34, 73.5%) were born and raised at the TNPRC. Another 7 (3 baboons and 4 pig-tailed macaques) of these NHP were born at the University of Washington Primate Center to mothers that had been born in captivity. One baboon was born in Kenya (where *T. cruzi* has never been reported) and lived for some time in Ohio, and the remaining NHP (a baboon) was born at the Yerkes Primate Center (Atlanta, GA) to a mother that had been born in captivity before both dam and offspring were transferred to the TNPRC.

More female (26 of 34, 76%) than male (8 of 34, 24%) NHP were infected, reflective of the sex bias in the population. The infected male-to-female ratio was 1:1 for rhesus macaques, 0:12 for pig-tailed macaques, and 1:7 for baboons. The Fisher exact test revealed no significant differences ($P > 0.05$) in infection prevalence between sexes for any of the 3 species examined. In addition, one mother–daughter pair was infected, suggesting the possibility of congenital transmission.

Discussion

In the current study, we report the low prevalence of *T. cruzi* infection in a NHP colony in Louisiana. Only 34 (1.6%) of 2157 NHP tested showed positive serology for *T. cruzi* by a dipstick assay. Further confirmation of the serologic test was provided by hemoculture of buffy coat from 4 NHP and by PCR amplification of a *T. cruzi*-specific band from the cultured parasites. Because the vast majority of the seropositive NHP (25 of 34, 73.5%) were captive-bred and raised in Louisiana; these findings strongly suggest local transmission of *T. cruzi*. Perhaps 2 of the 9 NHP born outside of Louisiana acquired the parasite in those other states, given that insect vectors or vectors and infected animals were reported in Ohio[32] and Georgia.[6,20] However, the 7 NHP born in Washington likely acquired the infection after their transfer to Louisiana, because *T. cruzi* has not been reported in that state. Infection was not evident by wet mount or Giemsa-stained blood smears in any of the samples, consistent with the low level of blood parasitemia in chronic infections.[28]

Local acquisition of *T. cruzi* by these NHP is not surprising, given that this colony is near (approximately 50 miles) the location of the sixth reported autochthonous human case in the

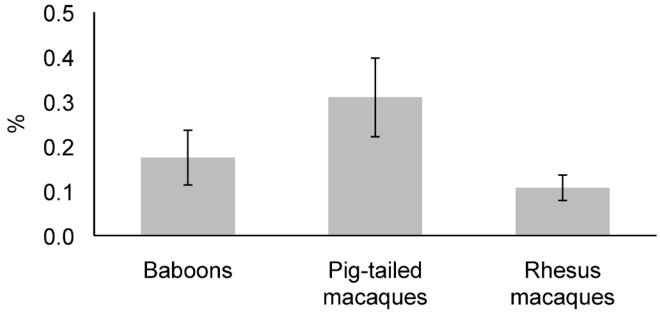

Figure 1. *Trypanosoma cruzi* infection prevalence for 3 species of nonhuman primates at TNPRC. Infection prevalence in pig-tailed macaques is significantly ($P < 0.05$) higher than that in rhesus macaques. Error bar, SEM.

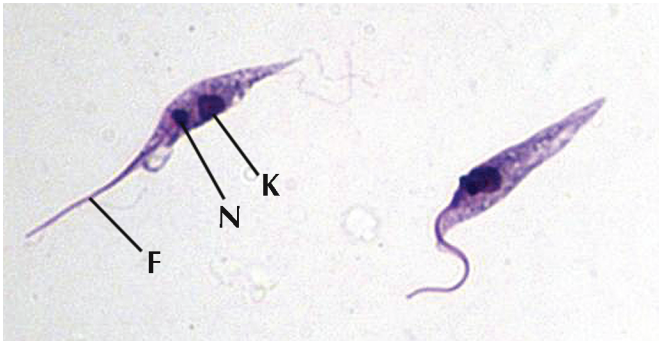

Figure 2. Giemsa-stained *T. cruzi* epimastigotes cultured from buffy coat of female *M. nemestrina*. k, kinetoplast; n, nucleus; f, flagellum.

United States[11] and is in an area known to have a high number of infected vectors and wild animals. Previous reports in Louisiana have shown that more than 50% of the local insect vector, *T. sanguisuga*, is infected.[8,11] In addition, in Louisiana, *T. cruzi* infection has been documented in 1.1% to 28.8% of armadillos,[3,34] 37.5% of opossums,[3] and 4.7% to 22.1% of dogs.[4,24] In some of these cases, the *T. cruzi* infection prevalence was higher than those found in countries with endemic infection in humans.[23] The difference in prevalence in armadillos and dogs is likely a real difference in infection prevalence among geographic localities and a reflection of the different sensitivities of the different detection methods used. Because the method used to measure *T. cruzi* prevalence in armadillos (hemoculture) is much less sensitive than is the dipstick assay used in the current study, our results suggest that infection levels in other mammals are greater than those seen in our NHP.

NHP are thought to contract the parasite by eating the insect vectors, in addition to known routes of transmission of the parasite for humans, such as contamination of the bite wound or mucous membranes. In fact, NHP in Georgia have been observed to handle and partially consume a triatomine bug.[27]

Unexpectedly, 35% of our infected NHP were pig-tailed macaques. This result is surprising, given that pig-tailed macaques comprised only 18% of the primates tested. In fact, pig-tailed macaques were infected significantly ($P < 0.05$) more often than were rhesus macaques but not baboons. Perhaps pig-tailed macaques are especially susceptible and therefore might be explored as an animal model for Chagas disease; limited studies to date have used NHP as model systems for Chagas disease.[2,35] In addition, the availability of local *T. cruzi* strains may be useful for Chagas studies using local NHP. Unfortunately, all but one cultured strain was lost in the aftermath of Hurricane Katrina; this strain could be useful for further studies on NHP, and additional strains could be cultured from the positive NHP. Of particular interest was the identification of a mother–offspring pair among the seropositive NHP. This finding suggests possibility of congenital transmission, an area of considerable interest in the Chagas community, and one that could be pursued in this colony.

The strong suggestion of local, ongoing transmission at this NHP colony has important implications for colony health. The low prevalence suggests that continuing modifications and control efforts (for example, removal of vegetation near cages, rodent control) are mostly effective; although it is unlikely all transmission can be avoided, given the high prevalence of infected insect vectors. In some previous reports of *T. cruzi* infection in NHP in the United States, the NHP were immunosuppressed and represent serendipitous findings that likely underestimate true prevalence.[9,17,25] When possible, monitoring *T. cruzi* infection in colonies in the Southern United States is recommended to understand the actual prevalence. The infection status of the NHP can affect the results of other studies, and infected animals could transmit the parasite to caregivers and lab workers, such as through contamination with infected blood.

In summary, the current study reports a low level of *T. cruzi* prevalence in a NHP colony in Louisiana. Chagas should be considered as a differential diagnosis for unexplained heart disease and it is important to continue the insect and pest control programs.

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