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Southern Plains Woodrats (*Neotoma micropus*) from Southern Texas Are Important Reservoirs of Two Genotypes of *Trypanosoma cruzi* and Host of a Putative Novel *Trypanosoma* Species

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Abstract

Trypanosoma cruzi, the causative agent of Chagas' disease, is an important public health and veterinary pathogen. Although human cases are rare in the United States, infections in wildlife, and in some areas domestic dogs, are common. In 2008 and 2010, we investigated *T. cruzi* prevalence in possible vertebrate reservoirs in southern Texas, with an emphasis on southern plains woodrats (*Neotoma micropus*). Infection status was determined using a combination of culture isolation, polymerase chain reaction (PCR), and serologic testing. Based on PCR and/or culture, *T. cruzi* was detected in 35 of 104 (34%) woodrats, 3 of 4 (75%) striped skunks (*Mephitis mephitis*), 12 of 20 (60%) raccoons (*Procyon lotor*), and 5 of 28 (18%) other rodents including a hispid cotton rat (*Sigmodon hispidus*), rock squirrel (*Otospermophilus variegatus*), black rat (*Rattus rattus*), and two house mice (*Mus musculus*). Additionally, another *Trypanosoma* species was detected in 41 woodrats, of which 27 were co-infected with *T. cruzi.* Genetic characterization of *T. cruzi* revealed that raccoon, rock squirrel, and cotton rat isolates were genotype TcIV, while woodrats and skunks were infected with TcI and TcIV. Based on the Chagas Stat-Pak assay, antibodies were detected in 27 woodrats (26%), 13 raccoons (65%), 4 skunks (100%), and 5 other rodents (18%) (two white-ankled mice [*Peromyscus pectoralis laceianus*], two house mice, and a rock squirrel). Seroprevalence based on indirect immunofluorescence antibody testing was higher for both woodrats (37%) and raccoons (90%), compared with the Chagas Stat-Pak. This is the first report of *T. cruzi* in a hispid cotton rat, black rat, rock squirrel, and white-ankled mouse. These data indicate that based on culture and PCR testing, the prevalence of *T. cruzi* in woodrats is comparable with other common reservoirs (i.e., raccoons and opossums) in the United States. However, unlike raccoons and opossums, which tend to be infected with a particular genotype, southern plains woodrats were infected with TcI and TcIV at near equal frequencies.

Key Words: Rodents—Triatomes—Trypanosomes—Zoonosis—Zoonotic.

Introduction

Trypanosoma cruzi, a flagellated protozoan parasite, is the etiologic agent of American trypanosomiasis or Chagas' disease. The parasite is primarily transmitted to vertebrate hosts in the feces of blood-sucking triatomine bugs, but infection of vertebrate hosts can also occur via blood transfusion, organ transplant, ingestion, lab accidents, or vertical transmission from mother to offspring (Hoff et al. 1978; Muños et al. 2007). Chagas' disease is endemic to the Americas and is a major cause of morbidity and mortality throughout Latin America with 8–11 million infected and ~50,000 deaths annually (Moncayo 1993; Centers for Disease Control and Prevention 2010). In the United States, only seven autochthonous cases have been reported in humans; four were from Texas, with one each from Louisiana, California, and Tennessee (Woody and Woody, 1955; Greer 1956; Ochs et al. 1996; Schiffler et al. 1984; Herwaldt et al. 2000; Dorn et al. 2007; Kjos et al. 2009). Chagas' disease is important from both a public health and veterinary perspective, since it can cause

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fatal myocarditis during the chronic stage in humans, domestic dogs, and some other vertebrates (e.g., baboons [*Papio papio*], macaques [*Macaca silenus*], polar bear [*Ursus maritimus*], and sugar gliders [*Petaurus breviceps*]; Jaime-Andrade et al. 1997; Pung et al. 1998; Latas et al. 2004; Williams et al. 2009).

The first report of *T. cruzi* in the U.S. was in a reduviid vector, *Triatoma protracta.* The bugs were collected from the nests of woodrats (*Neotoma* spp.) from San Diego, California (Kofoid, 1916). Approximately two decades later, *T. cruzi* was reported in the dusky-footed woodrat (*Neotoma fuscipes*) (Wood 1934). This was followed by detection of natural infections in other wildlife species in southern Texas, including Virginia opossums (*Didelphis virginiana*), nine-banded armadillos (*Dasypus novemcinctus*), and southern plains woodrats (*Neotoma micropus*) (Packchanian 1942). To date in the U.S., *T. cruzi* has been detected in more than 20 wildlife species, including several species of woodrats (*Neotoma* spp.). In addition to being hosts of *T. cruzi*, woodrats have been reported to be infected with at least two other *Trypanosoma* species, *T. neotomae* and *T. kansasensis* (Wood 1936; Wood and Wood 1937; Upton et al. 1989). These *Trypanosoma* spp. use fleas as intermediate hosts, whereas *T. cruzi* is transmitted by various species of reduviid bugs (Wood 1936).

Historically, hemoculture and examination of blood smears have been used to detect *T. cruzi* in wildlife in the U.S. (John and Hoppe, 1986). Recently, serological tests such as the indirect immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), and various rapid commercial tests (e.g., the Chagas Stat-Pak® assay; Chembio Diagnostic Systems, Inc., Medford, NY) have been utilized due to the low sensitivities of hemoculture and blood smear analysis for the detection of chronic infections. Rapid tests are reported to be of high sensitivity and specificity for detecting infections in humans, dogs, and some wildlife species, but these assays do not work on all species, and must be validated for use in new species (Partel and Rossi, 1998; Nieto et al. 2009; Yabsley et al. 2009).

Several studies have been conducted on the role of woodrats as reservoir hosts for *T. cruzi* (Eads and Hightower 1952; Eads et al. 1963; Pippin 1970; Burkholder et al. 1980; Pinto et al. 2010). It has been assumed that they are important reservoirs because they share nesting sites with reduviid vectors, and prevalence rates have been relatively high, ranging from 17–46% (Burkholder et al. 1980; Ikenga and Richerson 1984; Pinto et al. 2010). The primary aim of the current study was to examine potential reservoirs in Uvalde County, Texas, by determining the prevalence of *T. cruzi* in the southern plains woodrat and other known reservoir species using multiple diagnostic methods. Additionally, we genetically classified the samples of *T. cruzi* detected in woodrats and other hosts.

Materials and Methods

Trapping and blood collection

During July 2008 and March and May 2010, 104 southern plains woodrats (*Neotoma micropus*), 20 raccoons, four striped skunks, and 28 small rodents were trapped at a total of 4 sites in Uvalde County. Small rodents included 14 house mice (*Mus musculus*), 8 white-ankled mice (*Peromyscus pectoralis laceianus*), two hispid cotton rats (*Sigmodon hispidus*), a black rat (*Rattus rattus*), a white-footed mouse (*Peromyscus leucopus*), a rock squirrel (*Otospermophilus variegatus*), and a Mexican ground squirrel (*Ictidomys mexicanus*). Live traps (large Sherman traps; H.B. Sherman Traps, Tallahassee, Florida), and small squirrel cage traps (Havahart, Lititz, PA) were baited with dried apricots for rodents, and cage traps (Tomahawk Live Trap Co., Tomahawk, WI) were baited with sardines for raccoons and skunks. The traps were set during the afternoon and checked the following morning. Captured animals were weighed, anesthetized, and examined for ectoparasites. Rodents were anesthetized by intramuscular injection of 100 mg/kg of ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, IA). Skunks and raccoons were anesthetized using the same method with a mixture of 20 mg/kg ketamine (Fort Dodge Laboratories), and 4 mg/kg xylazine (Mobay Corporation, Shawnee, KS). Whole blood was collected by cardiocentesis from rodents and jugular venipuncture from skunks and raccoons and placed in potassium ethylenediaminetetraacetic acid (K_2EDTA) BD Vacutainer® tubes (Beckton Dickinson, Franklin Lakes, NJ). Rodents were euthanized by cervical dislocation and skunks and raccoons by intracardiac injection with an overdose of sodium pentobarbital (Butler Company, Columbus, OH), followed by exsanguination. All techniques were reviewed and approved by the IACUC at the University of Georgia.

Demographic parameters, including age and gender, were recorded for woodrats, skunks, raccoons, and other rodents. Raccoons and skunks were classified as adults or juveniles based on weight, tooth wear, and development of reproductive organs (Grau et al. 1970). Woodrats and other rodents were aged based on weight and development of reproductive organs.

Histopathology

Animals were necropsied and tissue samples (brain, lung, liver, heart, kidney, spleen, lymph nodes, quadriceps, gonads, and sections of the gastrointestinal tract) were preserved in 10% buffered formalin for histopathological examination. Small sections of formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for light microscopic examination. The sections were closely examined for the presence of amastigote nests.

Diagnostic methods for T. cruzi

Blood smears. Blood smears were prepared only from those woodrats trapped in 2010 (n = 56). Smears were made directly with fresh cardiac blood, air-dried, fixed in absolute alcohol for 5 min, and stained with Giemsa stain.

Hemoculture. A 1- to 10-mL aliquot of whole blood from raccoons, skunks, and larger rodents (woodrats, cotton rats, ground squirrel, and rock squirrel) was co-cultured in DH82 macrophages as previously described (Yabsley et al. 2004; Hall et al. 2007). Isolation attempts were not made for small rodents such as black rats, house mice, and white-ankled mice, due to insufficient blood volume. Briefly, to lyse erythrocytes, the blood was mixed with 35 mL of ammonium chloride potassium (ACK) lysing buffer (Quality Biological, Inc., Gaithersburg, MD), followed by gentle inversion for 5 min and centrifugation at 1620 g for 10 min. The supernatant was discarded and the process repeated. The resulting buffy coat was resuspended in 5 mL minimum essential media

(MEM) with 5% fetal bovine serum (FBS), and added to a confluent monolayer of DH82 cells. The culture was maintained at 37°C, fed twice weekly with fresh MEM supplemented with 5% FBS, and examined daily for the presence of trypomastigotes. If no parasites were evident after 6 weeks the sample was considered negative.

Serology. Two serologic assays for *T. cruzi,* the rapid Chagas Stat-Pak assay and an IFAT, were performed. The Chagas Stat-Pak assay was conducted according to the manufacturer's instructions using 5 μL serum or plasma. The IFAT was performed as previously described (Yabsley et al. 2001), with the following modifications. Antigen slides were made by placing mixed cultures of trypomastigotes of several *T. cruzi* strains (TcI and TcIV genotypes from the U.S.) on serology slides (Erie Scientific, Portsmouth, NH), which were then allowed to air dry and were subsequently fixed in acetone for 5 min. Serum/plasma samples diluted 1:40 with phosphate-buffered saline (PBS), and positive and negative controls, were incubated on the slides for 30 min at 37°C. This was followed by two 5-min washes of the slides with PBS, and a final wash with distilled water. The slides were then air dried. A commercial fluorescein (FITC-labeled) anti-species IgG antibody (1:25 dilution) was added to each well on the slides, and incubated for a further 30 min at 37°C. Secondary antibodies used for rodents were goat anti-mouse or anti-rat IgG (Kirkegaard and Perry Laboratories [KPL], Gaithersburg, MD). FITC-labeled goat anti-ferret and anti-raccoon IgG (KPL) were used for skunk and raccoon samples, respectively. Incubation dishes were covered with aluminum foil to prevent photo-bleaching of the fluorescein dye. The slides were washed twice in PBS for 5 min, and finally in a 1.65% Eriochrome Black T counterstain (Sigma-Aldrich, St. Louis, MO) in distilled water for 5 min. The slides were viewed under a Zeiss microscope equipped with a 50-watt Hg illuminator; the presence of green trypomastigotes indicated the sample was positive for *T. cruzi*, and red coloring was considered negative. Sera samples from laboratory-raised raccoons, laboratory-raised mice, laboratory-raised rats, and a wild-caught skunk (culture- and antibody-negative) were used as negative controls.

Molecular detection and characterization. DNA was extracted from 100 μL whole blood using the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA), according to the manufacturer's protocol. The extracted DNA was used as a template in a nested PCR, which amplified the D7 divergent domain of the 24Sα rDNA gene of *T. cruzi* using D75 5′-GCAGATCTT GGTTGGCGTAG-3′ and D76 5′-GGTTCTCTGTTGCCCCTT TT-3′ primers (Briones et al. 1999) in the primary reaction, followed by D71 5′-AAGGTGCGTCGACAGTGTGG-3′ and D72 5′-TTTTCAGAATGGCCGAACAGT-3′ primers in a secondary reaction (Souto et al. 1996). The samples were also tested for *T. cruzi* using the primers S35 5′-AAATAATGTACGGGTGGA GATGCATGA and S36 5′-GGGTTCGATTGGGGTTGGTGT, which target the kinetoplast minicircle DNA (Vallejo et al. 1999). DNA extractions, primary and secondary amplifications, and product analyses were performed in separate dedicated laboratory areas. A negative water control was also included in each set of DNA extractions and PCR reactions as contamination controls. The expected 125- or 110-bp 24Sα and 330-bp minicircle amplicons were visualized by trans-illumination of an ethidium bromide-stained 1.5% agarose gel. *T. cruzi* samples were classified in one of two lineages previously detected in the U.S. based on the 24Sα amplicons, which yields a 125-bp segment for TcI, or a 110-bp segment for TcIV (formally TcIIa; Souto et al. 1996). Representative amplicons from the D71 and D72 primer pair were sequenced at the University of Georgia Genomics Institute (Athens, Georgia) to confirm *T. cruzi* genotype results. Additionally, primary reactions that were positive, but were subsequently negative on the nested reaction with primers D71 and D72, were presumed to be the other *Trypanosoma* species we detected in these woodrats. Five random amplicons from these samples were sequenced to obtain phylogenetic information.

Statistical analysis

We used Fisher's exact test ($p = 0.05$ for significance) to test for differences in the prevalence rates of *T. cruzi* and the other *Trypanosoma* species by age, gender, diagnostic method, and host species (for *T. cruzi* only). Kappa statistics were used to compare the levels of agreement between the *T. cruzi* diagnostic tests.

Results

Of the 156 mammals tested from Uvalde County, 101 (65%) were infected with *T. cruzi* based on direct microscopic examination of blood smears, hemoculture, serology, or molecular testing. Trypomastigotes typical to *T. cruzi* were observed on stained blood films of only 3 of 56 woodrats (5%) sampled in 2010. These parasites measured between 15 and 17 μm in length, including the flagellum, and had a characteristic C- or S-shaped appearance. The kinetoplast was large and sub-terminal, giving the cell membrane a distorted appearance (Hoare 1972; Dusanic 1991).

Trypanosoma cruzi was isolated in DH82 cell cultures from 28 (18%) animals (Table 1). By species, *T. cruzi* was isolated from 18 woodrats (26%), 3 skunks (75%), 5 raccoons (25%), 1 rock squirrel (100%), and 1 hispid cotton rat (50%). Cultures from 35 woodrats were lost due to contamination with fungi and bacteria, despite the use of aseptic techniques during blood collection, and inclusion of antimicrobials and antifungals in culture media. Similar results were obtained with PCR testing, for which amplicons of the expected size for *T. cruzi* were generated from 23 of 104 woodrats (22%), 3 of 4 skunks (75%), 11 of 20 raccoons (55%), and 5 of 28 other rodents (18%; a cotton rat, rock squirrel, black rat, and two house mice; Table 1). A total of 25 of 104 (24%) woodrats were infected with the *T. neotomae*-like trypanosomes based on PCR, and 6 were co-infected with *T. cruzi* as determined by PCR (Table 2). Based on PCR, no significant difference in prevalence was noted between woodrats and other rodents, but prevalences in raccoons and skunks were significantly higher than in woodrats ($p = 0.005$ and $p = 0.043$, respectively).

Seventy-seven of 156 mammals (49%) had antibodies reactive with *T. cruzi* (Table 3). Based on combined results of the IFAT and Chagas Stat-Pak assay, 50 of the 104 woodrats (48%), all 4 skunks (100%), and 18 of 20 raccoons (90%) were seropositive. All of the small rodents were negative based on IFAT testing, but 5 (18%) of the small rodents (2 white-ankled mice, 2 house mice, and 1 rock squirrel) were positive for *T. cruzi* antibodies by the Chagas Stat-Pak assay. Seven of the 50 woodrats (14%) positive for *T. cruzi* by serology only were co-infected with the *T. neotomae*-like trypanosome (Table 2).

Table 1. Blood Smear, Hemoculture, and Polymerase Chain Reaction (PCR) Assay Results for *Trypanosoma cruzi* in 156 Mammals from Uvalde County, Texas

Species	Diagnostic assay No. positive/no. tested (%)			Total infected (%)
	Blood smear	Hemoculture	PCR	
Southern plains woodrat (*Neotoma micropus*) n=104	3/56 (5)	18/69 (26)	23/104 (22)	35/104 (34)
House mouse (*Mus musculus*) n=14	n.d.[b]	n.d.	2/14 (14)	2/14 (14)
White-ankled mouse (*Peromyscus pectoralis laceianus*) n=8	n.d.	n.d.	0/8 (0)	0/8 (0)
White-footed mouse (*Peromyscus leucopus*) n=1	n.d.	n.d.	0/1 (0)	0/1 (0)
Hispid cotton rat (*Sigmodon hispidus*) n=2	n.d.	1/2 (50)	1/2 (50)	2/2 (100)
Black rat (*Rattus rattus*) n=1	n.d.	n.d.	1/1 (100)	1/1 (100)
Mexican ground squirrel (*Ictidomys mexicanus*) n=1	n.d.	n.d.	0/1 (0)	0/1 (0)
Rock squirrel (*Otospermophilus variegatus*) n=1	n.d.	1/1 (100)	1/1 (100)	1/1 (100)
Raccoon (*Procyon lotor*) n=20	n.d.	5/20 (25)	11/20 (55)	12/20 (60)
Striped skunk (*Mephitis mephitis*) n=4	n.d.	3/4 (75)	3/4 (75)	3/4 (75)

n.d., not done.

Significantly more raccoons were seropositive compared with woodrats ($p=0.0004$) and other rodents ($p=0.0001$). No significant difference was noted between woodrats and skunks ($p=0.118$); however, woodrats had a significantly higher seroprevalence compared with other small rodents ($p=0.0046$). Overall, there was only a 55% agreement ($\kappa<0.20$) between the IFAT and Chagas Stat-Pak assays.

Another *Trypanosoma* species was detected in 41 of 104 (39%) woodrats (Table 2). This *Trypanosoma* species was observed in the blood smears of 3 of 56 (5%) woodrats (Table 2), but was most often observed in DH82 cultures (31 of 69 [45%] woodrats; Table 2). These parasites were observed in cell cultures for several days and slowly disappeared when the cells were fed fresh media. Giemsa-stained cytospins of scraped DH82 cells failed to show any intracellular stages of this *Trypanosoma* species; however, the parasites were frequently observed attached to the cell membranes. This *Trypanosoma* species failed to survive in liver infusion tryptose (LIT) media. No non-*T. cruzi* trypanosomes were observed in cultures from the skunks, raccoons, rock squirrel, or hispid cotton rat. Measurements of this woodrat *Trypanosoma* species were made from trypomastigotes observed in the blood smears of three woodrats. The total body length was 26.7 ± 3.15 (22–31 μm), the length of the body excluding the flagellum was 20.3 ± 2 (17–23 μm), free flagellar length was 5.61 ± 0.86 (4–7 μm), distance from end to kinetoplast was 4.9 ± 0.7 (4–6 μm), body width was 1.8 ± 0.43 (1–2.5 μm), nucleus length was 1.7 ± 0.37 (1.5–2.5 μm), kinetoplast length was 0.9 ± 0.18 (0.5–1 μm), and posterior end to nucleus was 13.7 ± 1.12 (12–15 μm). One woodrat had a mixed infection with this *Trypanosoma* species and *T. cruzi* on blood smear, and several other co-infections were noted based on culture and PCR testing (Table 2).

Histology

Only mild histopathologic lesions were observed in woodrats, and were primarily in skeletal muscle, heart, liver, and

Table 2. Results of Diagnostic Testing of Woodrats for a *Trypanosoma neotomae*-like Species and Association with *T. cruzi* Infection

	n	No. infected (% of total tested)	No. co-infected with T. cruzi based on PCR (% of infected) n=23	No. co-infected with T. cruzi based on culture (% of infected) n=18	No. co-infected with T. cruzi based on serology (% of infected)[a] n=50′
Blood smear	56	3 (5)	0 (0)	0 (0)	1 (2)
Culture	69	31 (45)	5 (22)	10 (56)	5 (10)
PCR	104	25 (24)	6 (26)	14 (78)	1 (2)
Total unique individuals		41 (39)	6 (26)	14 (78)	7 (14)

[a]These woodrats were only seropositive for *T. cruzi* and were PCR- and culture-negative.
PCR, polymerase chain reaction.

Table 3. Serology Results for *Trypanosoma cruzi* in 156 Mammals from Uvalde County, Texas

Species	*Chagas Stat-Pak assay* No. positive/no. tested (%)	*IFAT* No. positive/no. tested (%)	Total infected (%)
Southern plains woodrat (*Neotoma micropus*) n = 104	27/104 (26)	38/104 (37)	50/104 (48)
House mouse (*Mus musculus*) n = 14	2/14 (14)	0/14 (0)	2/14 (14)
White-ankled mouse (*Peromyscus pectoralis laceianus*) n = 8	2/8 (25)	0/8 (0)	2/8 (25)
White-footed mouse (*Peromyscus leucopus*) n = 1	0/1 (0)	0/1 (0)	0/1 (0)
Hispid cotton rat (*Sigmodon hispidus*) n = 2	0/2 (0)	0/2 (0)	0/2 (0)
Black rat (*Rattus rattus*) n = 1	0/1 (0)	0/1 (0)	0/1 (0)
Mexican ground squirrel (*Ictidomys mexicanus*) n = 1	0/1 (0)	0/1 (0)	0/1 (0)
Rock squirrel (*Otospermophilus variegatus*) n = 1	1/1 (100)	1/1 (100)	1/1 (100)
Raccoon (*Procyon lotor*) n = 20	13/20 (65)	18/20 (90)	18/20 (90)
Striped skunk (*Mephitis mephitis*) n = 4	4/4 (100)	4/4 (100)	4/4 (100)

IFAT, indirect immunofluorescent antibody test.

brain. Some lesions were characteristic of histopathology due to infection with other parasites such as *Hepatozoon* or *Sarcocystis* species. *T. cruzi* amastigote nests were not identified in any of the tissues examined.

Genetic characterization

Only two genotypes (TcI and TcIV) were detected among our isolates. Woodrats (10 TcI and 13 TcIV), and skunks (1 TcI and 3 TcIV), were infected with both genotypes, while all of the other positive rodents (hispid cotton rat and ground squirrel), and 5 raccoons, were infected with TcIV. Genotypes were not determined for all PCR-positive animals, since they could have been negative with D71/D72 primers, but positive with the S35/S36 primers.

Partial sequences (233 bp) of the 24Sα gene of the *T. neotomae*-like species from 5 woodrats were identical. Sequences were very similar to several rodent trypanosomes, including *T. kuseli* (99.1% identical, AB175626) from Siberian flying squirrels (*Pteromys volans*), *T. otospermophili* (99.1% identical, AB190225 and AB190228) from Richardson's and Columbian ground squirrels (*Urocitellus richardsonii* and *U. columbianus*, respectively), and *T. grosi* (98.7% identical, AB175624, AB175623, and AB175622) from *Apodemus speciosus* from Japan and *A. peninsualae* and *A. agrarius* from Vladivostok, Russia (Sato et al. 2005).

Population parameters

A total of 88 females (56 woodrats, 11 raccoons, 1 skunk, and 20 other rodents), and 68 males (48 woodrats, 9 raccoons, 3 skunks, and 8 other rodents) were tested in this study, of which 121 were adults (84 woodrats, 14 raccoons, 3 skunks, and 20 other rodents), and 28 were juvenile (20 woodrats, 6 raccoons, 1 skunk, and 1 other rodent). The ages of 7 rodents were undetermined. Although a slightly greater number of female woodrats (38 of 56) versus males (32 of 48) were PCR/culture-positive and seropositive, no significant difference was noted in the prevalence of *T. cruzi* by gender ($p = 1.000$). A similar trend was observed in skunks (1 female versus 3 males; $p = 1.000$), raccoons (11 females versus 7 males; $p = 0.190$), and other rodents (5 females versus 4 males; $p = 0.690$). No differences in prevalence were noted according to age for any of the species in this study.

Regarding the *Trypanosoma* species, there was no significant difference between male and female woodrats ($p = 0.842$; 21 of 55 females and 20 of 49 males), but significantly more adults compared with juveniles were infected ($p = 0.0113$; 38 of 83 adults and 3 of 21 juveniles).

Discussion

Rodents are common hosts for *T. cruzi* throughout the Americas, but little research has been done on prevalence rates in the U.S. To date, only 13 species have shown evidence of *T. cruzi* infection and only two isolates acquired from a single species (the southern plains woodrat) have been genetically characterized (Packchanian, 1942; Wood, 1952, 1962, 1975; Wood and Wood 1961; Burkholder et al. 1980; Navin et al. 1985; Roellig and Yabsley 2010). Many rodents, including woodrats, are insectivorous, and their burrows may be infested with *Triatoma* spp., which would increase exposure to *T. cruzi* (Eads et al. 1963; Ryckman et al. 1965; Wood and Wood 1976).

In the current study, using a combination of diagnostic assays, we detected a much higher prevalence of *T. cruzi* infection in southern plains woodrats (66%), compared with previous studies of the same species, which detected prevalence rates between 17 and 35% (Packchanian 1942; Eads and Hightower 1952; Pippin 1970; Burkholder et al. 1980; Pinto et al. 2010). One previous study (Ikenga and Richerson 1984)

found a prevalence rate of 46%; however, few woodrats were examined (n = 13), and the indirect hemagglutination (IHA) assay used for diagnosis of infections in that study has low specificity and reproducibility (Remesar et al. 2009). Although we detected a high prevalence of *T. cruzi* in woodrats, raccoons, and skunks, the animals sampled within the same county had significantly higher prevalence rates.

As expected, the combined prevalence rate of *T. cruzi* in woodrats based on hemoculture, blood smear, and PCR was lower (34%) than the serologic prevalence rate, since these diagnostic methods are most useful during acute infections, when relatively high parasitemias are present. Other surveillance studies conducted on woodrats utilizing blood smear and/or culture reported similar prevalence rates, ranging from 17–35% (Packchanian 1942; Eads and Hightower 1952; Burkholder et al. 1980). Similarly, our prevalence rate based on PCR of blood samples (22%) was comparable to a recent study (26%) that utilized PCR to survey tissue samples from museum specimens of southern plains woodrats (Pinto et al. 2010). Numerous studies have shown that serologic testing is more sensitive for detecting chronic infections with *T. cruzi,* since antibodies to *T. cruzi* can develop as early as 14 days post-infection, and positive titers persist in the circulation for years (Jansen et al. 1985; Dusanic 1991; Yabsley et al. 2001; Hall et al. 2010).

A *T. neotomae*-like species was also common in the southern plains woodrats. Based on morphological characteristics and measurements, this trypanosome was more similar to *T. neotomae* than *T. kansasensis. T. kansasensis* is easily distinguished from *T. neotomae* based on several morphologic measures, including a longer total body length (29.6–34 versus 22.5–33.7 μm), longer free flagellum length (6.4–11.2 versus 4.4–5.0 μm), and greater length between the posterior end and the kinetoplast (4.8–7.6 versus 2.7–4.4 μm; Davis 1952; Upton et al. 1989). The total length, free flagellar length, and distance from the posterior end to the kinetoplast of the southern plains woodrat *T*. species is similar to *T. neotomae*, but can be distinguished by a shorter distance from the posterior end to the kinetoplast, and shorter free flagellum length. No other measurements were available for *T. neotomae* for comparison. Several morphologic measures can distinguish our *Trypanosoma* species from *T. kansasensis*. Additionally, *T. kansasensis* has a significantly larger kinetoplast compared with our *Trypanosoma* species and *T. neotomae*. Previous studies have detected non-*T. cruzi* trypanosomes in *N. floridana* (eastern woodrat; *T. kansasensis,* 3 of 23, 13%), *N. micropus* (*T. neotomae,* 3 of 50, 6%), and *N. fuscipes* (*T. neotomae*, 1 of 78, 1.3%, and 12 of 61 (20%) (Wood, 1936; Packchanian 1942; Upton et al. 1989). All are believed to be transmitted by fleas, which were found in our study (Wood 1936; Hoare 1972). In general, prevalence rates of these trypanosomes were low compared to our study, in which 39% were infected with the *T. neotomae*-like species. Also of interest was the relatively high rate of co-infection of *T. neotoma*-like species infection in woodrats (27 of 41, 66%) with *T. cruzi.* Hemoculture proved to be the most efficient method of detecting co-infections of *T. cruzi* and the *T. neotomae*-like trypanosome, but it should be noted that a number of cultures were lost due to contamination, which led to a number of diagnoses being based on PCR. We believe that this *Trypanosoma* species from the southern plains woodrat may be novel, since some morphologic characteristics were different from those of *T. neotomae*; however, further work is needed to characterize this species, since our partial 24Sα gene sequence is the only sequence available from *Trypanosoma* spp. and from *Neotoma*.

The detection or isolation of *T. cruzi* from a hispid cotton rat, a black rat, two white-ankled mice, and a rock squirrel, represent new species reports. We also detected a high prevalence (32%) of *T. cruzi* among non-woodrat rodents when multiple diagnostic methods were used, compared to previous studies (5–16%), that were based on only one diagnostic assay (i.e., blood smear analysis, xenodiagnosis, or culture; Packchanian 1942; Burkholder et al. 1980; Navin et al. 1985). We suspect that the lower prevalence of *T. cruzi* among these rodents compared with woodrats is related to the biology of the woodrats and triatomine vectors. The close association of woodrats and triatomines is well documented (Kofoid 1916; Burkholder et al. 1980); however, little is known about triatomine interactions with other rodents. In addition, none of the rodents were positive with the IFAT assay, which could be due to a lack of cross-reactivity of our rodent antibody. The only seropositive non-woodrat rodents were detected with the Chagas Stat-Pak assay, which has been used to detect antibodies in experimentally-infected degus (*Octodon degus*), laboratory rats, and laboratory mice; however, this assay did not have very high sensitivity (Yabsley et al. 2009).

Although raccoons are commonly exposed to and infected with *T. cruzi*, the seroprevalence (90%) detected in raccoons from Uvalde County was higher than in all previous studies, even when individual locations are examined (maximum of 70% at one site in northern Florida; Brown et al. 2010; Maloney et al. 2010). Previous work in Arizona and the states east of the Mississippi River found seroprevalence rates between 20% and 20–68%, respectively (Burkholder et al. 1980; Yabsley and Noblet 2002; Hancock et al. 2005; Brown et al. 2010; Maloney et al. 2010). Within Texas, two previous studies have investigated *T. cruzi* in raccoons; one isolated *T. cruzi* from blood samples from 6 of 25 (24%) raccoons in Brown County (Schaffer et al. 1978), and another study failed to detect antibodies in 9 raccoons from southern Texas (Cameron and Hidalgo Counties) using an IHA assay (Burkholder et al. 1980). The high seroprevalence in seen southern Texas is likely related to increased host-vector interactions, as there is a high diversity and density of vectors in this region (Woody and Woody 1961; Kjos et al. 2009).

Results of the current and previous studies suggest that skunks are important hosts for *T. cruzi*. All four striped skunks we tested were positive for antibodies to *T. cruzi*. Although our sample size was small, previous studies, also with low sample sizes, have found similarly high seroprevalence rates: 3 of 34 (9%) from Arizona, 1 of 1 from California, and 1 of 1 from Georgia (Ryan et al. 1985; Brown et al. 2010). Striped skunks are omnivorous but consume a large number of insects (Monty and Emerson 2003). Additional work on the prevalence of *T. cruzi* among skunk populations is warranted.

In the United States, only two (TcI and TcIV) of the six known genotypes have been detected in vectors and vertebrate hosts (Roellig et al. 2008). A host-genotype dichotomy has been observed, with Virginia opossums being naturally and experimentally infected only with TcI, while raccoons are predominantly infected with TcIV, although they are susceptible to natural and/or experimental TcI infections (Clark and Pung 1994; Roellig et al. 2008; Roellig et al. 2010). To date,

all characterized autochthonous human infections with *T. cruzi* in the U.S. have been TcI (Roellig et al. 2008), which is the predominant cause of Chagas' disease north of the Amazon basin and Mexico (Bosseno et al. 2002). Interestingly, in the current study no predominant genotype was noted in woodrats; TcI accounted for 43% of isolates and TcIV accounted for 57%. Previously, only two southern plains woodrat *T. cruzi* isolates had been typed, and one was a TcI and the other was a TcIV (Roellig and Yabsley 2010). Previously, only a single isolate of *T. cruzi* from a striped skunk from Georgia had been typed, and it was a TcIV (Brown et al. 2010); our data indicate that skunks can be hosts for both TcI and TcIV genotypes. As has been found in previous studies, TcIV was the predominant genotype detected in raccoons (Roellig et al. 2008). The finding of TcIV in the cotton rat and a rock squirrel represents the first genetic characterization of rodent isolates of *T. cruzi,* except for two previously characterized southern plains woodrat isolates (Roellig and Yabsley 2010).

In summary, our data indicate that woodrats, raccoons, skunks, and other rodents are reservoirs of *T. cruzi* in Uvalde County, Texas, and that multiple diagnostic methods should be employed to acquire the best estimate of *T. cruzi* infection status in hosts. We also report *T. cruzi* infection in a rock squirrel, black rat, two white-ankled mice, and a hispid cotton rat, for the first time. In our study area, the data suggest that woodrats are a significant reservoir. Although the prevalence rates were similar to or lower than some other hosts in the area (e.g., raccoons and skunks), woodrats are present at high densities, and their nests are frequently infested with triatomines. Genetic characterization data suggest that woodrats can become infected with both TcI and TcIV genotypes with similar frequency (Roellig and Yabsley 2010). We also report the first infection of a striped skunk with a TcI isolate. Unfortunately no Virginia opossums, the most common host of TcI in the United States in previous studies, were captured during our field work. These alternative placental hosts may be important reservoirs of TcI in southern Texas in areas where opossum densities are not as high as other hosts. Further work on infectivity and virulence studies should be done on the two genotypes found in this study, combined with a larger geographic survey of woodrats as reservoirs of *T. cruzi* in the U.S. Additionally, isolates of *T. cruzi* should be genetically characterized to determine if other woodrat species or woodrats in other regions of the U.S. or Mexico are common hosts to multiple genotypes. Of interest for this species reservoir is the high rate of co-infections with another species of trypanosome, which is not observed in other common *T. cruzi* reservoirs in the U.S. Additional studies on the interactions of these two species are warranted to determine if infection dynamics are altered based on previous or concurrent infections.

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Author Disclosure Statement

No competing financial interests exist for any author.

References

Bosseno MF, Barnabe C, Magallon Gastelum E, et al. Predominance of *Trypanosoma cruzi* lineage I in Mexico. J Clin Microbiol 2002; 40:627–632.

Briones MRS, Souto RP, Stolf BS, et al. The evolution of two *Trypanosoma cruz*i subgroups inferred from rRNA genes can be correlated with the interchange of American mammalian faunas in the Cenozoic and has implications to pathogenicity and host specificity. Mol Biochem Parasitol 1999; 104:219–232.

Brown EL, Roellig DM, Gompper ME, et al. Seroprevalence of *Trypanosoma cruzi* among eleven potential reservoir species from six states across the southern United States. Vector Borne Zoonotic Dis 2010; 10:757–763.

Burkholder JE, Allison TC, Kelly VP. *Trypanosoma cruzi* (Chagas) (Protozoa, Kinetoplastida) in invertebrate, reservoir, and human hosts of the lower Rio Grande Valley of Texas. J Parasitol 1980; 66:305–311.

Centers for Disease Control and Prevention. Parasites—American trypanosomiasis (also known as Chagas' disease), 2010, http://www.cdc.gov/parasites/chagas/

Clark CG, Pung OJ. Host specificity of ribosomal DNA variation in sylvatic *Trypanosoma cruzi* from North America. Mol Biochem Parasitol 1994; 66:175–179.

Davis BS. Studies on the trypanosomes of some California mammals. Univ Calif Publ Zool 1952; 57:145–249.

Dorn PL, Perniciaro L, Yabsley MJ, et al. Autochthonous transmission of *Trypanosoma cruzi,* Louisiana. Emerg Infect Dis 2007; 13:605–607.

Dusanic DG. *Trypanosoma (Schizotrypanum) cruzi.* In: *Parasitic Protozoa.* Kreier JP, Baker JR, ed. New York: Academic Press, 1991:137–194.

Eads RB, Hightower BG. Blood parasites of southwest Texas rodents. J Parasitol 1952; 38:89–90.

Eads RB, Trevino HA, Campos EG. *Triatoma* (Hemiptera: Reduviidae) infected with *Trypanosoma cruzi* in south Texas wood rat dens. Southwest Nat 1963; 8:38–42.

Grau GA, Sanderson GC, Rogers JP. Age determination of raccoons. J Wildl Manage 1970; 34:364–372.

Greer DA. Found: two cases of Chagas' disease. Tex Health Bull 1956; 9:11–13.

Hall CA, Pierce EM, Wimsatt AN, et al. Virulence and vertical transmission of two genotypically and geographically diverse isolates of *Trypanosoma cruzi* in mice. J Parasitol 2010; 96: 371–376.

Hall CA, Polizzi C, Yabsley MJ, et al. *Trypanosoma cruzi* prevalence and epidemiologic trends in lemurs on St. Catherines Island, Georgia. J Parasitol 2007; 93:93–96.

Hancock K, Zajac AM, Pung OJ, et al. Prevalence of antibodies to *Trypanosoma cruzi* in raccoons (*Procyon lotor*) from an urban area of northern Virginia. J Parasitol 2005; 91:470–472.

Herwaldt BL, Grijalva MJ, Newsome AL, et al. Use of polymerase chain reaction to diagnose the fifth reported US case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, 1998. J Infect Dis 2000; 181:395–399.

Hoare CA. The stercoraria. In: *The Trypanosomes of Mammals. A Zoological Monograph.* Oxford: Blackwell Scientific Publications, 1972:327–400.

Hoff R, Mott KE, Milanesi ML, et al. Congenital Chagas' disease in an urban population: Investigation of infected twins. Trans R Soc Trop Med Hyg 1978; 72:247–250.

Ikenga JO, Richerson JV. *Trypanosoma cruzi* (Chagas') (protozoa: Kinetoplastida: Trypanosomatidae) in invertebrate and vertebrate hosts from Brewster County in Trans-Pecos Texas. J Econ Entomol 1984; 77:126–129.

Jaime-Andrade J, Avila-Figueroa D, Lozano-Kasten FJ, et al. Acute Chagas' cardiopathy in a polar bear (*Ursus maritimus*) in Guadalajara, Mexico. Rev Soc Bras Med Trop 1997; 30:337–340.

Jansen AM, Moriearty PL, Castro BG, Deane MP. *Trypanosoma cruzi* in the opossum (*Didelphis marsupialis*), an indirect fluorescent antibody test for the diagnosis and follow-up of natural and experimental infections. Trans R Soc Trop Med Hyg 1985; 79:474–477.

John DT, Hoppe KL. *Trypanosoma cruzi* from wild raccoons in Oklahoma. Am J Vet Res 1986; 47:1056–1059.

Kjos SA, Snowden KF, Olson JK. Biogeography and *Trypanosoma cruzi* infection prevalence of Chagas' disease vectors in Texas, USA. Vector Borne Zoonotic Dis 2009; 9:41–49.

Kofoid CA, McCullock I. On *Trypanosoma triatomae*, a new flagellate from a hemipteran bug from the nests of the wood rat; *Neotoma fuscipes*. Univ Calif Pub Zool 1916; 15:113–126.

Latas P, Reavill DR, Nicholson D. *Trypanosoma* infection in sugar gliders (*Petaurus breviceps*) and a hedgehog (*Atelerix albiventris*) from Texas. Joint conference-American Association of Zoo Veterinarians 2004; 183–184.

Maloney J, Newsome A, Huang J, et al. Seroprevalence of *Trypanosoma cruzi* in raccoons from Tennessee. J Parasitol 2010; 96:353–358.

Moncayo A. Chagas' disease. Tropical disease research: eleventh programme report, World Health Organization. Geneva, Switzerland, 1993:62–75.

Monty AM, Emerson RE. Eastern wood rat. In: *Wild Mammals of North America : Biology, Management, and Conservation.* Feldhamer GA, Thompson BC, Chapman JA, ed. Baltimore: Johns Hopkins University Press, 2003:381–393.

Muños J, Portus M, Corachan M, et al. Congenital *Trypanosoma cruzi* infection in a non-endemic area. Trans R Soc Trop Med Hyg 2007; 101:1161–1162.

Navin TR, Roberto RR, Juranek DD, et al. Human and sylvatic *Trypanosoma cruzi* infection in California. Am J Public Health 1985; 75:366–369.

Nieto PD, Boughton R, Dorn PL, et al. Comparison of two immunochromatographic assays and the indirect immunofluorscence antibody test for diagnosis of *Trypanosoma cruzi* infection in dogs in south central Louisiana. Vet Parasitol 2009; 165:241–247.

Ochs DE, Hnilica VS, Moser DR, et al. Postmortem diagnosis of autochthonous acute chagasic myocarditis by polymerase chain reaction amplification of a species-specific DNA sequence of *Trypanosoma cruzi*. Am J Trop Med Hyg 1996; 54:526–529.

Packchanian A. Reservoir hosts of Chagas' disease in the state of Texas. Natural infection of nine-banded armadillo (*Dasypus novemcinctus texanus*), house mice (*Mus musculus*), opossum (*Didelphis virginiana*), and wood rats (*Neotoma micropus micropus*), with *Trypanosoma cruzi* in the state of Texas. Am J Trop Med Hyg 1942; 22:623–631.

Partel CD, Rossi CL. A rapid, quantitative enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of Chagas' disease. Immunol Invest 1998; 27:89–96.

Pinto CM, Baxter BD, Hanson JD, et al. Using museum collections to detect pathogens. Emerg Infect Dis 2010; 16:356–357.

Pippin WF. The biology and vector capability of *Triatoma sanguisuga texana* (Usinger) and *Triatoma gerstaeckeri* (Stal) compared with *Rhodnius prolixus* (Stal) (Hemiptera: Triatominae). J Med Entomol 1970; 7:30–45.

Pung OJ, Spratt J, Clark CG, Norton TM, et al. *Trypanosoma cruzi* infection of free-ranging lion-tailed macaques (*Macaca silenus*) and ring-tailed lemurs (*Lemur catta*) on St. Catherine's Island, Georgia, USA. J Zoo Wildl Med 1998; 29:25–30.

Remesar MC, Gamba C, Colaianni IF, et al. Estimation of sensitivity and specificity of several *Trypanosoma cruzi* antibody assays in blood donors in Argentina. Transfusion 2009; 49:2352–2358.

Roellig DM, Brown EL, Barnabe C, et al. Molecular typing of *Trypanosoma cruzi* isolates, United States. Emerg Infect Dis 2008; 14:1123–1125.

Roellig DM, McMillan K, Ellis AE, et al. Experimental infection of two South American reservoirs with four distinct strains of *Trypanosoma cruzi*. Parasitology 2010; 137:959–966.

Roellig DM, Yabsley MJ. Short report: infectivity, pathogenicity, and virulence of *Trypanosoma cruzi* isolates from sylvatic animals and vectors, and domestic dogs from the United States in ICR strain mice and SD strain rats. Am J Trop Med Hyg 2010; 83:519–522.

Ryan CP, Hughes PE, Howard EB. American trypanosomiasis (Chagas' disease) in a striped skunk. J Wildl Dis 1985; 21:175–176.

Ryckman RE, Folkes DL, Olsen LE, et al. Epizootiology of *Trypanosoma cruzi* in southwestern North America. I: new collection records and hosts for *Trypanosoma cruzi* Chagas (Kinetoplastida: Trypanosomidae) (Hemiptera: Triatominae). J Med Entomol 1965; 2:87–108.

Sato H, Osanai A, Kamiya H, et al. Characterization of SSU and LSU rRNA genes of three *Trypanosoma* (*Herpetosoma*) *grosi* isolates maintained in Mongolian jirds. Parasitology 2005; 130:157–167.

Schaffer GD, Hanson WL, Davidson WR, et al. Hematotropic parasites of translocated raccoons in the southeast. J Am Vet Med Assoc 1978; 173:1148–1151.

Schiffler RJ, Mansur GP, Navin TR, et al. Indigenous Chagas' disease (American trypanosomiasis) in California. J Am Med Assoc 1984; 251:2983–2984.

Souto RP, Fernandes O, Macedo AM, et al. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. Mol Biochem Parasitol 1996; 83:141–152.

Upton SJ, Fridell RA, Tilley M. *Trypanosoma kansasensis* sp. n. from *Neotoma floridana* in Kansas. J Wildl Dis 1989; 25:410–412.

Vallejo GA, Guhl F, Chiari E, et al. Species specific detection of *Trypanosoma cruzi* and *Trypanosoma rangeli* in vector and mammalian hosts by polymerase chain reaction amplification of kinetoplast minicircle DNA. Acta Trop 1999; 72:203–212.

Williams JT, Dick EJ, VandeBerg JL, et al. Natural Chagas' disease in four baboons. J Med Primatol 2009; 38:107–113.

Wood FD. Natural and experimental infection of *Triatoma protracta* Uhler and mammals in California with American human trypanosomiasis. Am J Trop Med Hyg 1934; 14:497–511.

Wood FD. *Trypanosoma neotomae*, sp. nov., in the dusky-footed wood rat and the wood rat flea. Univ Calif Publ Zool 1936; 41:133–143.

Wood FD, Wood SF. Occurrence of haematozoa in some California birds and mammals. J Parasitol 1937; 23:197–201.

Wood SF. Blood parasites of mammals of the California Sierra Nevada foothills, with special reference to *Trypanosoma cruzi* and *Hepatozoon leptosoma* sp. n. Bull South Calif Acad Sci 1962; 61:161–176.

Wood SF. Mammal blood parasite records from southwestern United States and Mexico. J Parasitol 1952; 38:85–86.

Wood SF. *Trypanosoma cruzi*: new foci of enzootic Chagas' disease in California. Exp Parasitol 1975; 38:153–160.

Wood SF, Wood FD. Ecological notes on Chagas' zoonosis in New Mexico. Pan-Pac Entomol 1976; 4:348–350.

Wood SF, Wood FD. Observations on vectors of Chagas' disease in the United States. III. New Mexico. Am J Trop Med Hyg 1961; 10:155–165.

Woody NC, Woody HB. American trypanosomiasis (Chagas' disease), 1st indigenous case in the United States. JAMA 1955; 159:676–677.

Woody NC, Woody HB. American trypanosomiasis. 1. Clinical and epidemiologic background of Chagas disease in United States. J Pediatr 1961; 58:568–580.

Yabsley MJ, Brown EL, Roellig DM. Evaluation of the Chagas Stat-Pak™ assay for detection of *Trypanosoma cruzi* antibodies in wildlife reservoirs. J Parasitol 2009; 95:775–777.

Yabsley MJ, Noblet GP, Pung OJ. Comparison of serological methods and blood culture for detection of *Trypanosoma cruzi* infection in raccoons (*Procyon lotor*). J Parasitol 2001; 87:1155–1159.

Yabsley MJ, Noblet GP. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. J Wildl Dis 2002; 38:75–83.

Yabsley MJ, Norton TM, Powell MR, et al. Molecular and serologic evidence of tick-borne ehrlichiae in three species of lemurs from St. Catherines Island, Georgia, USA. J Zoo Wildl Med 2004; 35:503–509.

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