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Identification of Bloodmeal Sources and *Trypanosoma cruzi* Infection in Triatomine Bugs (Hemiptera: Reduviidae) From Residential Settings in Texas, the United States

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Abstract

The host–vector–parasite interactions in Chagas disease peridomestic transmission cycles in the United States are not yet well understood. *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) infection prevalence and bloodmeal sources were determined for adult and immature triatomine (Hemiptera: Reduviidae) specimens collected from residential settings in central Texas. Sequenced cytochrome *b* DNA segments obtained from triatomine digestive tract identified nine vertebrate hosts and one invertebrate host in four triatomine species (*Triatoma gerstaeckeri*, *Triatoma indictiva*, *Triatoma protracta*, and *Triatoma sanguisuga*). The broad range of wild and domestic host species detected in triatomine specimens collected from residential sites indicates high host diversity and potential movement between the sylvatic and peridomestic settings. Domestic dogs appear to be key in the maintenance of the peridomestic transmission cycle as both a blood host for the triatomine vectors and a potential reservoir for the parasite. The high rate of *T. cruzi* infection among triatomine specimens that were collected from inside houses, outside houses, and dog kennels (69, 81, and 82%, respectively) suggests a current risk for Chagas disease vector-borne transmission for humans and domestic animals in residential settings in Texas because of overlap with the sylvatic cycle.

Keywords

triatomine; *Trypanosoma cruzi*; host association; bloodmeal analysis

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Chagas disease, caused by the hemoflagellate protozoan parasite, *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), is the most important parasitic disease on the American continents, accounting for an estimated 8 million human infections, with 56,000 new cases and 12,000 deaths annually occurring in Latin American countries (PAHO 2006, World Health Organization [WHO] 2008). Hematophagous triatomine bugs (Hemiptera: Reduviidae: Triatominae) are the biological arthropod vectors of *T. cruzi*, transmitting the parasite in infectious feces deposited on or near the host during or shortly after the bug feeds. The parasite enters the vertebrate host when the feces contaminate the bite site or mucous membranes. Although vector-borne transmission continues to be the primary route of infection, alternative modes of transmission include blood transfusion, organ transplantation, congenital, and oral ingestion of contaminated foods or drinks (Coura and Dias 2009). Enzootic transmission cycles are endemic across the southern United States, where 24 wildlife mammalian species have been identified as reservoirs, acute and chronic infections have been documented in at least 48 domestic dog breeds, and triatomine vectors have been reported from 28 states (Bern et al. 2011).

Of the 141 currently recognized triatomine species, 11 are native to the United States and all are considered potential vectors of Chagas disease (Schofield and Galvao 2009). Although 8 of the 11 species have been associated with human bites in the United States (Packchanian 1940, Wood 1942, Arnold and Bell 1944, Ryckman 1981, Klotz et al. 2010), human *T. cruzi* infection because of autochthonous vector-borne transmission has been rarely reported. Between 1955 and 2006, seven human cases were reported in two adults and five young children from the states of Texas ($n = 4$), CA ($n = 1$), Tennessee ($n = 1$), and Louisiana ($n = 1$) (Woody and Woody 1955, Anonymous 1956, Schiffler et al. 1984, Ochs et al. 1996, Herwaldt et al. 2000, Dorn et al. 2007, Kjos et al. 2009). With the exception of one adult case from Louisiana, all infected individuals sought medical attention because of acute symptoms. Since 2007, voluntary testing of donated blood for *T. cruzi* antibodies in the United States has identified >1,600 confirmed seropositive donations (AABB 2012). Although most seropositive donors are Latin American immigrants who likely acquired the infection before relocating to the United States, a subset of these seropositive donors were U.S.-born with no travel history to endemic areas (Bern et al. 2008). A recent study of confirmed seropositive U.S. blood donors identified 16 additional autochthonous cases, all asymptomatic chronic infections, presumably acquired by vector-borne transmission in the United States (Cantey et al. 2012). The predominant risk factors included living in a southern state where triatomine vectors or infected mammalian reservoirs had been reported and participation in outdoor activities. Documentation of human–triatomine encounters in the United States predates the discovery of the *T. cruzi* vector-borne transmission cycle (Leconte 1855, Kimball 1894), and these encounters continue to be reported today primarily in association with allergic reactions to triatomine bites (Klotz et al. 2010, Reisenman et al. 2010). The frequency of human–triatomine encounters in the United States is considerably lower than in areas with high vector-borne transmission rates in Latin America, presumably because of the uncommon occurrence of triatomine colonization of U.S. houses. However, the compelling evidence for vector-borne transmission in newly diagnosed chronic cases among blood donors suggests vector-borne transmission may occur more frequently in the United States than previously recognized, particularly because acute infection is very mild or asymptomatic in most immunocompetent individuals (Rassi et al. 2010).

Whereas autochthonous human *T. cruzi* infections in the United States are rarely reported, acute and chronic cases among domestic dogs have been documented widely across the southern states, beginning with Texas in 1972 (Williams et al. 1977, Tomlinson et al. 1981, Barr et al. 1991, Duprey et al. 2006, Kjos et al. 2008, Rowland et al. 2010). The disparity in reported infection prevalence between humans and dogs in the United States is likely because of differences in vector exposure levels and transmission routes, as well as an

increasing awareness among veterinarians of the potential risk of infection for dogs and therefore increased testing (Kjos et al. 2008) in contrast to the lack of awareness of human risk among physicians (Stimpert and Montgomery 2010, Verani et al. 2010). The presence of infected domestic animals in peridomestic and domestic settings, particularly dogs, is a significant contributor to human infection in certain areas of South America, with dogs serving both as blood hosts for the triatomine vectors and as parasite reservoirs (Gürtler et al. 1986, 1996, 2007). Dogs housed in proximity to two U.S. human autochthonous cases tested positive for *T. cruzi* antibodies, providing some evidence of a potential role for dogs in human risk in the United States (Navin et al. 1985, Herwaldt et al. 2000). Recent studies of *T. cruzi* infection in triatomine specimens collected in and around houses in the United States report prevalence rates of 19% (n = 139) in California (Hwang et al. 2010), 42% (n = 164) in Arizona (Reisenman et al. 2010), 53% (n = 137) in Texas (Kjos et al. 2009), and 56% (n = 18) in Louisiana (Dorn et al. 2007), indicating continued presence of infected vectors in peridomestic and domestic domains across a wide geographic area. The interactions of hosts, parasite, and vectors in the United States in the peridomestic and domestic domains, where triatomine species are not habitually domiciliated, are not yet well understood.

Bloodmeal analysis has proven effective for elucidating vector–host interactions and pathogen transmission dynamics in vector-borne disease systems, including, malaria, Lyme disease (Pichon et al. 2003), leishmaniasis (Haouas et al. 2007), African trypanosomiasis (Muturi et al. 2011), several arboviral diseases (Molaei et al. 2006, Scott et al. 2006), and Chagas disease (Zarate et al. 1980). Originally based on serological methods, bloodmeal analysis in arthropod vectors has largely shifted to DNA-based techniques that offer greater accessibility by eliminating the need for host-derived antisera, and increased specificity in host identification (Mukabana et al. 2002, Kent 2009). The use of bloodmeal analysis in triatomine vectors has been informative in determining host preferences and seasonal changes in host selection (Gürtler et al. 1997), describing disparities in *T. cruzi* infection rates among triatomine populations (Villalobos et al. 2011), analyzing movement of triatomine vectors between different habitats (Pizarro and Stevens 2008), and assessing the vector potential of a species (Sandoval et al. 2004). The application of bloodmeal analysis to U.S. triatomine species has only recently been reported in a study of 13 specimens collected using light traps in sylvatic settings in California in which rodent, canine, avian, porcine, and human DNA were detected (Stevens et al. 2012). The aim of the current study was to determine hosts that serve as bloodmeal sources and to quantify *T. cruzi* infection levels of triatomine species found in residential settings in Texas, particularly those associated with domestic dogs. Identification of the sylvatic and domestic hosts used by triatomine specimens collected in this study will also provide information on the components of the transmission cycle in this area as well as triatomine movement between the sylvatic and domestic settings. Our overall objective was to better understand the current vector-borne transmission risk in this Chagas disease endemic region of the United States.

Materials and Methods

Experimental Evaluation of Bloodmeal Analysis Polymerase Chain Reaction Assays

The feasibility of using previously published polymerase chain reaction (PCR) assays for bloodmeal analysis was evaluated in triatomine bugs. Two species of laboratory-reared bugs, *Triatoma dimidiata* and *Rhodnius prolixus*, were fed defibrinated blood from two blood hosts, human and rabbit, and tested at different time intervals using a mammal-specific assay (Molaei et al. 2006) and a vertebrate-specific assay (Boakye et al. 1999) (Table 1). Bugs were fed rabbit blood at all previous feedings, with the last meal before the experiment occurring during the previous instar. Bugs were used for the experiment 2–4 wk

after their last molt. Bugs were fed to repletion on a single source of blood using an artificial membrane feeding system (Pennington et al. 2004). Seven fourth-instar *T. dimidiata* and eight fifth-instar *R. prolixus* nymphs were fed human blood, and one specimen of each species was tested on days 0, 5, 10, 20, 31, 40, 55, and 69. Nine fourth-instar *T. dimidiata* and nine fifth-instar *R. prolixus* nymphs were fed rabbit blood, and one specimen of each species was tested on days 4, 9, 16, 20, 25, 30, 42, 60, and 71.

Specimen Collection and Processing

Specimens were collected during the summer and fall of 2008 at eight residential sites in five counties located in central Texas (Uvalde County, sites 1–4; Zavala County, site 5; Comal County, site 6; Bexar County, site 7; Gonzales County, site 8). Property sizes were either ≈3 acres and located in an urban setting (site 6), or were >10 acres and located in rural settings (all other sites). Dogs were present at seven residences (sites 1–7), three of which had a history of canine Chagas disease (sites 3, 5, and 6). Other animals housed within the peridomestic setting at the sites include cats (sites 1–4 and 8), cattle (sites 2, 3, and 5), horses (sites 1 and 4–7), goats (sites 3 and 4), poultry (sites 2,4, and 7), and pigs (site 7). All residents reported previous presence of triatomines on the site, four reported presence inside houses (sites 3, 4, 7, and 8), and two reported human bites (sites 7 and 8). Manual inspection of each site was conducted from 7 July 2008 to 19 August 2008 for 2–4 d during both daylight and nighttime hours, including inside houses and surrounding areas, dog kennels, outbuildings, wood and rock piles, tree cavities, and yard lights. Woodrat nests located at three rural sites in Uvalde County (sites 1, 3, and 4) were dismantled and searched. A universal black light funnel trap (BioQuip Products, Rancho Dominguez, CA) was placed in a natural flyway protected from yard and house lights on each property for two to three nights. Captured bugs were placed in separate ventilated containers and cooled on ice until processed. Additional triatomine specimens collected by residents of the sites from 23 June 2008 to 20 November 2008 were submitted directly to the laboratory.

Location, date, stage, sex (adults only), and condition (live/dead) were recorded at the time of collection. Adult specimens were identified to the species level using the taxonomic key of Lent and Wygodzinsky (1979). Nymphs were identified to the species level using molecular methods described later in the text. Qualitative nutritional status of adult specimens was determined by direct observation of the backlit anterior midgut and classified as either starved, or with scarce, good, or large volume of blood (Ceballos et al. 2005, Vazquez–Prokopec et al. 2006). For nymphs, a qualitative nutritional classification of either starved or not starved was assigned based on presence or absence of abdominal distention. Specimens that were completely desiccated at the time of processing were excluded. For specimens collected during field studies, the digestive tract was removed (Garcia and De Azambuja 1997) using sterile forceps and scalpel, and placed in 50 μl phosphate-buffered saline. Samples were flash-frozen in an ethanol + dry ice bath and stored on dry ice until transported to the laboratory, where they were stored at –70°C until processed. For specimens submitted directly to the laboratory, the lower abdomen was detached with a sterile disposable scalpel and homogenized with a 0.25-inch sterile ceramic bead for 20 s using the BIO101/Savant FastPrep FP120 (Qbiogene, Inc., Carlsbad, CA). DNA extraction was performed using the QIAmp DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA), according to the manufacturer's instructions.

Molecular Analysis

The primer sequences, cycling parameters, and references for the PCR assays are provided in Table 1. All PCR products were visualized using electrophoresis on 1.5% agarose gels, stained with ethidium bromide. PCR products subject to sequencing were purified using the Millipore MultiScreen$_{HTS}$ PCR 96-well plate system (Millipore Corp., Billerica, MA), and

sequenced using the BigDye Terminator Ready Reaction Kit v1.1 and ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). To minimize the risk of cross-contamination among samples, DNA extraction, PCR setup, and electrophoresis were conducted in separate dedicated areas of the laboratory.

Triatomine Species Identification of Nymphs

Standard PCR methods were used to amplify fragments of the mitochondrial cytochrome *b* (*cytb*) gene (Monteiro et al. 2003) or D2 section of the nuclear 28S ribosomal RNA gene (Fitzpatrick et al. 2008) (Table 1). Sequences that were unreadable using the *cytb* assay were amplified and sequenced using an internal segment of the *cytb* fragment (Table 1). Sequences were compared with previously generated sequences from morphologically identified adult specimens to determine species.

T. cruzi Detection

Two DNA targets were amplified to determine infection status: *T. cruzi*-specific TCZ1/2 nuclear satellite repeat (Moser et al. 1989) and Tc121/122 minicircle fragment (Wincker et al. 1994) (Table 1). A positive result as determined by visualization of the expected band size on an agarose gel for both assays was required for a specimen to receive a positive *T. cruzi* infection status.

Bloodmeal Analysis

Three different *cytb* targets were used in three separate PCR assays to identify bloodmeal hosts belonging to mammalian, avian, or vertebrate animal groups. Two of the targets, primer sets Mammalian a F/R (Molaei et al. 2006) and L15557/H16065 (Cicero and Johnson 2001) (Table 1), specifically amplify mammal and bird DNA, respectively. The third target, primer set BM1/BM2 (Boakye et al. 1999) (Table 1), amplifies all vertebrate DNA, including reptiles and amphibians (Meece et al. 2005). Positive DNA controls were used in each assay as follows: macaw (avian), dog (mammalian and vertebrate), and ground skink (vertebrate). Sterile water was used as a negative control in all assays. The products of all positive reactions were sequenced and compared with DNA nucleotide sequences in NCBI BLAST using the megablast query. Query results achieving ≥95% maximum identity and Expect (E) value ≤0 were considered matches. Samples matching field cricket DNA were subjected to a fourth PCR assay targeting a field cricket-specific mitochondrial cytochrome *c* oxidase subunit one (COI) fragment (Simon et al. 1994, Gray et al. 2008) (Table 1).

Statistical Analysis

All statistical tests were performed in SAS 9.2 software (SAS Institute Inc., Cary, NC). Chi-square tests were used to assess differences in probability of obtaining a bloodmeal identification by nutritional status (starved vs. not starved) and state at dissection (live vs. dead). The nutritional status of adult specimens was converted to either starved or not starved (with scarce, good, or large volume of blood) to allow for the combination of adults and nymphs in this analysis. Specimens with unreadable or undetermined sequences only were removed from the χ^2 analysis.

Results

Evaluation of Bloodmeal Analysis PCR Assays

The laboratory-based feeding experiments resulted in different PCR detection limits for the two tested assays by both blood source and triatomine species (Table 2). The vertebrate assay detected human blood for a longer period postfeeding (up to day 55) compared with the mammalian assay (up to day 40), and for an equal number of days in the two triatomine

species. The assays performed the same for specimens fed rabbit blood, with a longer period postfeeding detectable in *T. dimidiata* compared with *R. prolixus.* Rabbit blood was detectable the greatest number of days postfeeding (to at least day 71) compared with human blood (up to day 55).

Triatomine Specimen Collections by Site

In total, 153 triatomine specimens were collected and processed (site 1, $n = 50$; site 2, $n = 2$; site 3, $n = 10$; site 4, $n = 13$; site 5, $n = 9$; site 6, $n = 53$; site 7, $n = 10$; site 8, $n = 6$) (Table 3). Inspection of 59 woodrat nests yielded 60 specimens of two species (*T. protracta*, adults, $n = 4$, nymphs, $n = 45$; *T. gerstaeckeri*, nymphs, $n = 11$) from nine nests at two of the three sites where woodrat nests were searched (Table 3). All triatomine specimens were collected by manual search methods or submitted by residents; none were captured using the black light funnel trap. The specimens were primarily collected from woodrat nests ($n = 60$, 39%) and dog kennels ($n = 59$, 39%), with the remaining specimens collected from around ($n = 18$, 12%) and inside houses ($n = 16$, 10%).

Specimens of five triatomine species were collected, and 78% of specimens tested were infected with *T. cruzi* (Table 3). The most commonly collected species was *T. gerstaeckeri* ($n = 70$, 46%). The DNA target of the two PCR assays used for triatomine species identification did not provide enough sequence differentiation between the morphologically identified *T. sanguisuga* and *T. indictiva* adult specimens, and no sequences for *T. indictiva* were available in GenBank for comparison. Thus we were unable to make a definite species determination for 21 fifth-instar nymphs, which could be either *T. sanguisuga* or *T. indictiva.* Because the two species are sympatric in our study area, these specimens were designated as *T. sanguisuga/indictiva* for the analyses. Sequences generated for species identification have been submitted to GenBank (KF188579-663).

Identification of Bloodmeal Hosts

A bloodmeal host was identified for 63% ($n = 96$) of the specimens (Table 4). Identification of a bloodmeal host was significantly higher in specimens not starved (83/99, 84%) compared with specimens that were starved (12/36, 33%; $\chi^2 = 32.30$, df = 1, $P < 0.0001$). Identification of a bloodmeal host was significantly higher in specimens alive at the time of dissection (55/70, 79%) compared with specimens dead at the time of dissection (41/67, 61%; $\chi^2 = 4.93$, df = 1, $P = 0.026$).

Nine vertebrate and one invertebrate host species were identified in four species of triatomine bugs (Table 4). Dog DNA was detected in three triatomine species and was collected from the greatest number of sites ($n = 4$). Woodrat was the most frequently detected vertebrate species ($n = 47$), followed by dog ($n = 19$), cat ($n = 6$), cow ($n = 5$), and one each for turkey vulture, black vulture, human, raccoon, and pig. One invertebrate species, field cricket, was detected in 15 nymphs using the vertebrate assay. Nine of the 15 samples produced amplicons using the field cricket-specific COI assay. No reptile or amphibian hosts were identified. The greatest variety of host species was identified in *T. gerstaeckeri* (nine), including mammal, bird, and insect species. Woodrat was the only host species identified in *T. protracta* specimens that were exclusively collected from woodrat nests. One mixed bloodmeal was identified in an adult *T. sanguisuga* (dog and turkey vulture) collected in Comal County (site 6).

The vertebrate and mammalian assays differed in results by species detected and frequency of detection. The vertebrate assay detected pig and raccoon DNA in a single specimen each, but the mammalian assay was negative for these same specimens. The mammalian assay detected woodrat DNA in 47 specimens, but the vertebrate assay detected woodrat DNA in

only six of these specimens. The mammalian assay also more frequently detected dog and cat DNA compared with the vertebrate assay (18 vs. 3, 6 vs. 1, respectively). The vertebrate and avian assays differed in results with the vertebrate assay only detecting one of the two bird species detected by the avian assay. Seventeen successfully sequenced PCR products (n = 8, vertebrate assay; n = 9, avian assay) failed to match with at least 95% identity to an available reference sequence in GenBank, so they were considered "undetermined." Twenty-seven PCR products had unreadable sequences (n = 25, vertebrate assay; n = 2, mammalian assay). The vertebrate assay amplified human DNA in 21 specimens, of which only one was confirmed with the mammalian assay. Whereas seven of these samples produced negative PCR results by the other assays, 13 produced a different host species. From these, 10 specimens were identified as containing woodrat DNA by the mammalian assay. These specimens were all nymphs collected from woodrat nests, which makes it highly unlikely that they had fed on humans because of their limited mobility (wingless in the immature stages) and distant location from houses. Because of this fact and the lack of agreement between assays, all human results obtained only with the vertebrate assay were disregarded. PCR and sequencing results by assay for each specimen are provided in Appendix A.

Discussion

Bloodmeal hosts were identified in four species of Chagas disease vectors collected in central Texas and included seven mammalian, two avian, and one invertebrate species. Four of the seven groups of domestic animals (dogs, cats, cattle, and pigs) known to be housed at the study sites were detected through bloodmeal analysis. Woodrat DNA was the only host detected in specimens collected from woodrat nests. Raccoon and both vulture species identified in specimens are commonly found in the study area (Benson and Arnold 2001, Schmidly 2004). Human DNA was detected in one adult specimen, *T. gerstaeckeri*, collected from a bedroom in Bexar County (site 7), where residents had reported recent triatomine bites.

Based on the laboratory feeding experiments, the success of host identification postfeeding using the vertebrate and mammalian assays ranged from 20 d to at least 71 d, varying with both triatomine and host species. These results were similar to other studies in which postfeeding detection limits using a *cytb* marker ranged from 30 to 70 d (Bosseno et al. 2006, Mota et al. 2007). Although determination of assay sensitivity in triatomine bugs was beyond the scope of this study, we observed that the vertebrate assay proved to be much more sensitive in detecting human DNA in the laboratory feeding experiments, particularly in specimens tested at greater number of days postfeeding and in specimens that had molted. The shorter amplicon produced by the vertebrate primers compared with the mammalian primers may make amplification more successful in bloodmeals where the DNA has degraded with time and digestive processing or contain only trace amounts. These results suggest that assay sensitivity can vary by both host and triatomine species, as well as by postfeeding period, possibly because of variation in bloodmeal processing time among triatomine species and stages and differences in blood components among host species. Furthermore, field specimens with higher nutritional status (evidence of meal in digestive tract) and those alive at the time of processing were more likely to have a bloodmeal host identified using these methods.

Although the PCR assays used in this study proved successful in achieving our goals and have been cited extensively in bloodmeal analysis in other insect vectors (Ngo and Kramer 2003, Kilpatrick et al. 2006, Molaei et al. 2006, Richards et al. 2006, Savage et al. 2007, Hamer et al. 2008), limitations were noted. Mixed bloodmeals were detected in <1% (1/153) of specimens in this study, compared with 6.4% (11/64) and 40.7% (33/81) in specimens

collected from peridomestic settings in Mexico and Bolivia, respectively, using other nondirect sequencing PCR-based host detection methods (Bosseno et al. 2006, Pizarro and Stevens 2008). The ability to detect multiple host species in a single specimen was limited by direct sequencing of a single product from each assay. In addition, 18% (27/153) of specimens had a PCR positive result but unreadable sequences, which could be because in part of the presence of DNA from multiple host species. Cloning and sequencing multiple samples from individual specimens may have increased our ability to detect multiple hosts (Stevens et al. 2012), but were not conducted because of cost and time limitations. DNA sequencing using the *cytb* gene, which has extensive taxonomic coverage in GenBank, was determined to be the optimal approach for this study because the host range for the bug species of interest is unknown. Even so, 17 samples produced high-quality sequences but did not satisfactorily match with any published material in GenBank. With definitive knowledge of a set of host species (10 species identified through our current methods), considerations for future studies in this geographic area may include adaptations to enhance detection of multiple bloodmeal sources and reduce costs for large sample sizes through the use of alternative DNA-based methods such as terminal restriction fragment length polymorphism, heteroduplex, or high-resolution melting assays (Meece et al. 2005, Buitrago et al. 2012, Pena et al. 2012).

The disparity in results from the bloodmeal identification assays may be because of differences in sensitivity or PCR bias, which has been reported in other gene markers (Polz and Cavanaugh 1998, Acinas et al. 2005). Differences between the vertebrate and the mammalian assays, including the identification of human DNA by the vertebrate assay but not the mammalian assay in the same samples, have been previously reported (Murdock et al. 2010). We questioned the validity of human positive results obtained by the vertebrate assay because of the lack of agreement between the vertebrate and mammalian results and the fact that most of these human results came from nymphs captured in woodrat nests, which have a low dispersal probability, particularly as first and second instars. Whereas little data have been reported on the active dispersal capabilities of triatomine nymphs, fifthinstar nymphs collected from light traps were estimated to have traveled up to 42 m from the nearest known infested sites in a study in Argentina (Vazquez–Prokopec et al. 2004). In our study, the distance from the woodrat nests to houses was considerable: 46–114 m at one site and 109 m at the other site. The lack of a blood host in the nest does not appear to be a reason for dispersal because other specimens collected from the same nests were positive for woodrat DNA and these human positive specimens by the vertebrate assay were positive for woodrat DNA by the mammalian assay. In addition, the woodrat nests from which the specimens were collected had signs of recent activity and woodrats were noted to be present at the sites (Charles et al. 2013), further corroborating the woodrat DNA-positive results. If an adult triatomine had fed on a human host and then returned to the woodrat nest, it is plausible that the nymphs ingested human blood while feeding on the engorged adult (Wood and Wood 1941, Ryckman 1951, Hays 1965, Alves et al. 2011). In this study, no adult specimens collected from woodrat nests or any other location at these two sites were positive for human DNA. Contamination could be considered a potential factor, even though all DNA extraction and PCR negative controls were negative and DNA extractions and PCR setup were performed in separate designated areas. The errant detection of human DNA under carefully controlled laboratory conditions has been reported in PCR-based bloodmeal studies in other arthropods (Humair et al. 2007).

The detection of invertebrate DNA (field cricket) by the vertebrate assay indicates the occurrence of nonspecific binding, which has been reported previously in this marker (Murdock et al. 2010). Identification of field cricket DNA was confirmed using the field cricket-specific COI assay. The lack of COI amplification in 6 of the 15 specimens may be because of lower sensitivity of the COI assay compared with the vertebrate *cytb* assay.

Although we were unable to distinguish between the closely related field cricket species, *Gryllus texensis* and *Gryllus rubens*, based on sequence comparisons in GenBank, the species is most likely *G. texensis*, the Texas field cricket, based on the known geographic distribution of the two *Gryllus* species (Gray et al. 2008) and the collection site of our samples. The 15 field cricket-positive specimens were among 28 fourth- and fifth-instar nymphs collected from beneath a concrete slab located in a dog kennel (site 6). The practice of triatomines feeding on the hemolymph of other arthropods (hemolymphagy), including cockroaches and spiders, has been previously reported (Miles et al. 1981, Sandoval et al. 2004). The collection site of the field cricket-positive nymphs was within 3 m of kenneled dogs. One other nymph collected with the field cricket-positive specimens at this same site was positive for dog DNA. The stage (fifth instar) and species (*T. sanguisuga* and *T. indictiva*) of the dog-positive nymph were also the predominant species and stage of the field cricket-positive nymphs. It appears this triatomine species is able to readily switch between vertebrate and invertebrate hosts in the immature stages, but the circumstances that influence the selection of hosts are unknown. Because the assays used in this study were designed to identify vertebrate blood host species, we cannot rule out the possibility of the presence of other invertebrate blood host species in these specimens.

The high rate of *T. cruzi* infection among triatomine specimens collected from inside houses, outside houses, and in dog kennels (69, 81, and 82%, respectively) suggests a current risk for Chagas disease vector-borne human and domestic animal transmission in residential settings in central Texas. The methods used in this study do not allow us to directly associate the bloodmeal host species with the infection source of *T. cruzi* of a given specimen because we could, presumably, only identify the most recent bloodmeal. Infection could have occurred during a previous meal, which may have been from a different host species. In addition, we are not able to conclude host preferences for a particular triatomine species because host species abundance and availability at the time of feeding are unknown for most host species. However, we can conclude that the triatomine species collected in this study willingly feed on the hosts identified, and the high *T. cruzi* prevalence represents a risk for infection to the susceptible host species. Data on the prevalence of *T. cruzi* infection in wild mammals are available from a related study for three of the study sites (1, 3, and 4) plus one additional site in Uvalde County (Charles et al. 2013). Briefly, a high *T. cruzi* seroprevalence was detected in woodrats (50/104, 48%), skunks (four-fourths, 100%), raccoons (18/20, 90%), and other rodents (5/28, 18%) sampled at the sites in 2008 and 2010. Combined, these results indicate that *T. cruzi* is currently circulating in both sylvatic and peridomestic cycles in this area of Texas.

Domestic dogs, particularly those confined to outdoor kennels, appear to be key in the maintenance of the peridomestic transmission cycle in Texas as both a blood host for the triatomine vectors, as determined in this study, and potentially a reservoir for the parasite. Previous research indicates that *T. cruzi* infection among dogs in Texas occurs more frequently in the sporting and working breeds, which are more likely to be housed in outdoor kennels (Kjos et al. 2008). Two of the four sites where dog DNA was detected in triatomine specimens had a known history of canine Chagas disease. The dogs at these sites (Labrador Retriever, German Shorthaired Pointer, and Pointer) were used for bird hunting and were housed in confined outside kennels. Dogs at the remaining two dog DNA-positive sites were housed outside, unconfined, indicating that sleeping outdoors (confined or unconfined) puts dogs at risk for encounters with triatomine vectors. Although the risk of Chagas disease has been reported to be greater for dogs sleeping outdoors compared with indoors in a study in urban Mexico (Jimenez–Coello et al. 2010), other factors including triatomine species, *T. cruzi* prevalence, and dog behavior may impact infection risk among dogs that sleep outside.

Although both dogs and cats have been implicated in peridomestic and domestic transmission cycles in South America as both a blood host and parasite reservoir (Gürtler et al. 1996, 2007), data are lacking on the role of cats in U.S. transmission cycles. To our knowledge, this is the first report of U.S. triatomine species using cats as a bloodmeal host. Additional research on infectivity of dogs and cats to triatomine vectors and pathogenicity of strains in triatomine vectors collected in residential settings in the United States would be beneficial to further understanding the dynamics and potential threat of Chagas disease vector-borne transmission cycles operating in proximity to humans.

The broad range of wild and domestic host species detected in triatomine specimens collected from residential sites indicates high host diversity and potential movement between the sylvatic and peridomestic settings. In addition to the 10 host species described here, analysis of additional triatomine specimens from Texas using the same methods included detection of chimney swift in *T. sanguisuga* (Brazos County) and nine-banded armadillo and striped skunk in *T. gerstaeckeri* (Bexar and Nueces County, respectively) (S.A.K., unpublished data). Flexibility in host selection could complicate efforts to control or eliminate the bugs from residential property in the study area and subsequently pose a risk for *T. cruzi* transmission. At one site in Comal County, 7 of 15 dogs housed on the property were serologically positive for *T. cruzi* at the time of the study. The previous summer, the acute death of a 6-yr-old Pointer belonging to the residents was attributed to *T. cruzi* infection based on histopathologic evidence, making it the first known canine case in the kennel. It was also the first year the residents noticed the presence of triatomine bugs on their property, although they had resided and housed dogs on the property for 13 yr. The residence is located in a rapidly growing urban area north of the city of San Antonio (population 1,327,407). A comparison of aerial photographs taken the first year the residents occupied the residence and the year of the study (13 yr later) shows pervasive human development in the surrounding region over that period. The displacement of wild vertebrate hosts in the area because of habitat changes may have facilitated the movement of triatomine bugs to the residence, where the kenneled dogs provided an easily accessible alternative bloodmeal source. Human alteration of natural habitat and subsequent migration of triatomine bugs to residential settings has been reported for *T. protracta* in California (Wood and Wood 1964) as well as for several species in deforested areas of Central and South America (Romana et al. 2003, Aguilar et al. 2007). The confirmed persistence of *T. cruzi* and low specificity of triatomine host selection in this area of Texas, coupled with the dynamic nature of residential settings, present a challenge for both predicting and preventing *T. cruzi* transmission among humans and their companion animals.

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Appendix

Appendix A

Bloodmeal identification results by PCR assay

Site–specimen	*Triatoma* species[a]	Stage[b]	PCR assay		
			Vertebrate	Mammalian	Avian
1–1	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–2	*p*	N4	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–3	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–4	*g*	N4	Not determined[c]	*Neotoma micropus*	Negative PCR
1–5	*p*	N1	Not determined[c]	*Neotoma micropus*	Negative PCR
1–6	*p*	N1	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–7	*p*	N2	Not determined[c]	*Neotoma micropus*	Negative PCR
1–8	*p*	N2	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–9	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–10	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–11	*p*	N3	Not determined[c]	*Neotoma micropus*	Negative PCR
1–12	*p*	N4	Not determined[c]	*Neotoma micropus*	Negative PCR
1–13	*p*	N4	Not determined[c]	Negative PCR	Negative PCR
1–14	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–15	*p*	N4	Unreadable sequence	Unreadable sequence	Negative PCR
1–16	*p*	N4	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–17	*p*	N4	Not determined[c]	*Neotoma micropus*	Negative PCR
1–18	*p*	N1	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–19	*p*	N5	Negative PCR	Negative PCR	Negative PCR
1–20	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–21	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–22	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–23	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–24	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–25	*p*	N4	Negative PCR	Negative PCR	Negative PCR
1–26	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–27	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–28	*p*	A	Not determined[c]	Negative PCR	Negative PCR
1–29	*p*	N3	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–30	*p*	N4	Negative PCR	Unreadable sequence	Negative PCR
1–31	*p*	N5	Negative PCR	*Neotoma micropus*	Negative PCR
1–32	*g*	N5	Negative PCR	Negative PCR	Negative PCR
1–33	*p*	N5	Unreadable sequence	Negative PCR	Negative PCR
1–34	*p*	N5	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
1–35	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–36	*p*	N4	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–37	*p*	N5	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–38	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR

Site–specimen	*Triatoma* species[a]	Stage[b]	PCR assay		
			Vertebrate	Mammalian	Avian
1–39	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–40	*p*	N5	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
1–41	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–42	*p*	N4	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–43	*p*	N5	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–44	*p*	N4	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
1–45	*p*	N4	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
1–46	*p*	N5	*Homo sapiens*	*Neotoma micropus*	Negative PCR
1–47	*p*	A	Negative PCR	Negative PCR	Negative PCR
1–48	*p*	N5	Unreadable sequence	*Neotoma micropus*	Negative PCR
1–49	*p*	A	Negative PCR	*Neotoma micropus*	Negative PCR
1–50	*p*	A	Unreadable sequence	Negative PCR	Negative PCR
2–1	*g*	A	Negative PCR	*Felis catus*	Negative PCR
2–2	*g*	A	Negative PCR	*Felis catus*	Negative PCR
3–1	*g*	N4	*Homo sapiens*	*Neotoma micropus*	Negative PCR
3–2	*g*	N3	Negative PCR	Negative PCR	Negative PCR
3–3	*p*	N1	*Homo sapiens*	*Neotoma micropus*	Negative PCR
3–4	*g*	N2	Unreadable sequence	*Neotoma micropus*	Negative PCR
3–5	*g*	N2	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
3–6	*g*	N2	*Homo sapiens*	*Neotoma micropus*	Negative PCR
3–7	*g*	N1	Negative PCR	Negative PCR	Negative PCR
3–8	*g*	N2	*Homo sapiens*	*Neotoma micropus*	Negative PCR
3–9	*g*	N2	*Neotoma micropus*	*Neotoma micropus*	Negative PCR
3–10	*g*	N1	Negative PCR	Negative PCR	Negative PCR
4–1	*g*	A	Unreadable sequence	Negative PCR	Negative PCR
4–2	*l*	A	Unreadable sequence	Negative PCR	Negative PCR
4–3	*g*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
4–4	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–5	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–6	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–7	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–8	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–9	*g*	A	*Homo sapiens*	Negative PCR	Negative PCR
4–10	*g*	N5	*Homo sapiens*	*Canis lupus familiaris*	Not determined[c]
4–11	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–12	*g*	A	Negative PCR	Negative PCR	Negative PCR
4–13	*g*	A	Negative PCR	Negative PCR	Negative PCR
5–1	*g*	A	Negative PCR	*Bos taurus*	Negative PCR
5–2	*g*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
5–3	*g*	N5	Negative PCR	*Canis lupus familiaris*	Negative PCR
5–4	*g*	A	Negative PCR	Negative PCR	Negative PCR

Site–specimen	*Triatoma* species[a]	Stage[b]	PCR assay		
			Vertebrate	Mammalian	Avian
5–5	*g*	A	Negative PCR	*Bos taurus*	Negative PCR
5–6	*g*	A	Negative PCR	*Bos taurus*	Negative PCR
5–7	*g*	A	Negative PCR	*Bos taurus*	Negative PCR
5–8	*g*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
5–9	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–1	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–2	*g*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
6–3	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–4	*s*	A	*Homo sapiens*	Negative PCR	Negative PCR
6–5	*g*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
6–6	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–7	*s*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
6–8	*s*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
6–9	*g*	A	*Homo sapiens*	Negative PCR	Negative PCR
6–10	*s*	A	*Homo sapiens*	*Canis lupus familiaris*	*Cathartes aura*
6–11	*g*	A	*Procyon lotor*	Negative PCR	Negative PCR
6–12	*g*	A	*Sus scrofa*	Negative PCR	Negative PCR
6–13	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–14	*i*	A	Negative PCR	Negative PCR	Negative PCR
6–15	*g*	A	Negative PCR	*Bos taurus*	Negative PCR
6–16	*i*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
6–17	*s*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
6–18	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–19	*s*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
6–20	*i*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
6–21	*s*	A	*Canis lupus familiaris*	*Canis lupus familiaris*	Negative PCR
6–22	*g*	A	Unreadable sequence	*Felis catus*	Not determined[c]
6–23	*g*	A	*Coragyps atratus*	Negative PCR	*Coragyps atratus*
6–24	*g*	A	Negative PCR	Negative PCR	Negative PCR
6–25	*g*	A	Negative PCR	*Canis lupus familiaris*	Negative PCR
6–26	*g*	N5	Negative PCR	Negative PCR	Negative PCR
6–27	*g*	N5	Negative PCR	Negative PCR	Negative PCR
6–28	*g*	N5	Negative PCR	Negative PCR	Negative PCR
6–29	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–30	*s/i*	N5	Negative PCR	Negative PCR	Negative PCR
6–31	*g*	N5	Negative PCR	Negative PCR	Negative PCR
6–32	*g*	N4	Negative PCR	Negative PCR	Negative PCR
6–33	*s/i*	N5	Negative PCR	Negative PCR	Negative PCR
6–34	*g*	N4	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–35	*g*	N4	Negative PCR	Negative PCR	Negative PCR
6–36	*s/i*	N5	Negative PCR	Negative PCR	Negative PCR

Site–specimen	*Triatoma* species[a]	Stage[b]	PCR assay		
			Vertebrate	Mammalian	Avian
6–37	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–38	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–39	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–40	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–41	*s/i*	N5	Negative PCR	Negative PCR	Negative PCR
6–42	*s/i*	N5	Negative PCR	Negative PCR	Negative PCR
6–43	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–44	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–45	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–46	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–47	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–48	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–49	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–50	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–51	*s/i*	N5	*Gryllus texensis/rubens*	Negative PCR	Negative PCR
6–52	*s/i*	N5	negative PCR	Negative PCR	Negative PCR
6–53	*s/i*	N5	*Canis lupus familiaris*	Negative PCR	Negative PCR
7–1	*g*	A	Negative PCR	Negative PCR	Negative PCR
7–2	g	A	*Homo sapiens*	*Homo sapiens*	Negative PCR
7–3	*s*	A	*Homo sapiens*	Negative PCR	Negative PCR
7–4	*g*	A	Negative PCR	Negative PCR	Negative PCR
7–5	*g*	A	*Homo sapiens*	Negative PCR	Negative PCR
7–6	*g*	A	Negative PCR	Negative PCR	Negative PCR
7–7	*g*	A	*Canis lupus familiaris*	*Canis lupus familiaris*	Not determined[c]
7–8	*g*	A	Negative PCR	*Canis lupus familiaris*	Not determined[c]
7–9	*s*	A	Negative PCR	Negative PCR	Negative PCR
7–10	*g*	A	*Homo sapiens*	Negative PCR	Negative PCR
8–1	*g*	A	*Homo sapiens*	*Felis catus*	Negative PCR
8–2	*g*	A	Negative PCR	Negative PCR	Negative PCR
8–3	*g*	A	Unreadable sequence	Negative PCR	Negative PCR
8–4	*g*	A	*Felis catus*	*Felis catus*	Negative PCR
8–5	*g*	A	Negative PCR	*Felis catus*	Negative PCR
8–6	*g*	A	*Homo sapiens*	Negative PCR	Negative PCR

[a] *g, gerstaeckeri; i, indictiva; p, protracta; s, sanguisuga; s/i, sanguisuga* or *indictiva*.

[b] A, adult; N, nymph, no. refers to instar.

[c] BLAST query maximum identity < 95%, E value > 0.

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Table 1

Description of PCR assays

Target	Primer name	Sequence (5′-3′)	Product bp	Cycling conditions				Reference
				Denaturation	Annealing	Extension	No. cycles	
Bloodmeal analysis								
Vertebrate mtDNA *cytb*	BM1	CCC CTC AGA ATG ATA TTT GTC CTC A	358	95°C, 30 s	57°C, 50 s	72°C, 40 s	36	(Boakye et al. 1999)
	BM2	CCA TCC AAC ATC TCA GCA TGA TGA AA						
Mammalian mtDNA *cytb*	Mammalian a F	CGA AGC TTG ATA TGA AAA ACC ATC GTT	772	95°C, 30 s	55°C, 45 s	72°C, 1.5 min	36	(Molaei et al. 2006)
	Mammalian a R	TGT AGT T(A/G)T C(A/T)G GGT C(A/C/T)C CTA						
Avian mtDNA *cytb*	L15557	GAC TGT GAC AAA ATC CC(G/A/T/C) TTC CA	508	95°C, 30 s	57°C, 50 s	72°C, 40 s	36	(Cicero and Johnson 2001)
	H16065	GGT CTT CAT CT(C/T) (C/T/A)GG (T/C)TT ACA AGA C						
Cricket mtDNA COI	C1-J-2183	CAA CAT TTA TTT TGA TTT TTT GG	724	94°C, 30 s	52°C, 30 s	72°C, 1 min	30	(Simon et al. 1994, Gray et al. 2008)
	TL2-N-3014	TCC AAT GCA CTA ATC TGC CAT ATT A						
Trypanosoma cruzi detection								
nDNA satellite repeat	TCZ1	CGA GCT CTT GCC CAC ACG GGT GCT	188	94°C, 20 s	57°C, 10 s	72°C, 30 s	35	(Moser et al. 1989)
	TCZ2	CCT CCA AGC AGC GGA TAG TTC AGG						
Minicircle kDNA	Tc121	AAA TAA TGT ACG GG(T/G) GAG ATG CAT GA	330	95°C, 30 s	58°C, 30 s	72°C, 1 min	35	(Wincker et al. 1994)
	Tc122	GGT TCG ATT GGG GTT GGT GTA ATA TA						
Triatomine species identification								
mtDNA *cytb*	cytb7432F	GGA CG(AT) GG(AT) ATT TAT TAT GGA TC	663	94°C, 30 s	47°C, 30 s	72°C, 1 min	35	(Monteiro et al. 2003)
	cytb7433R	GC(AT) CCA ATT CA(AG) GTT A(AG)T AA						
	cytb-intF	(C/T)CT ACT ATC CGC GGT TCC TTA	443	95°C, 30 s	51°C, 50 s	72°C, 40 s	36	this paper
	cytb-intR	ATA CTA TTG CAA TTA CTC CTC CTA						
28S RNA D2	D2F	GCG AGT CGT GTT GCT TGA TAG TGC AG	633	94°C, 1 min	50°C, 2 min	72°C, 2 min	25	(Fitzpatrick et al. 2008)

Target	Primer name	Sequence (5′-3′)	Product bp	Cycling conditions				Reference
				Denaturation	Annealing	Extension	No. cycles	
	D2R	TTG GTC CGT GTT TCA AGA CGG G						

Table 2

Comparison of PCR assays in laboratory-reared triatomine bugs fed human or rabbit blood

Blood host	Days postfeeding	*Triatoma dimidiata*			*Rhodnius prolixus*		
		Stage	PCRm	PCRv	Stage	PCRm	PCRv
Human[a]	0	N4	+	+	N5	+	+
	5	N4	+	+	N5	+	+
	10	N4	+	+	N5	+	+
	20	N4	+	+	N5	+	+
	31	N4	+	+	Adult	–	+
	40	N4	+	+	Adult	–	+
	55	N4	–	+	Adult	–	+
	69	–	–	–	Adult	–	–
Rabbit[b]	4	N4	+	+	N5	+	+
	9	N4	+	+	N5	+	+
	16	N4	+	+	N5	+	+
	20	N4	+	+	N5	+	+
	25	N4	+	+	Adult	+	+
	30	N4	+	+	Adult	–	–
	42	N4	+	+	Adult	–	–
	60	N5	+	+	Adult	–	–
	71	N5	+	+	–	–	–

PCRm, mammalian a F/R; PCRv, vertebrate BM1/BM2.

All *T. dimidiata* and *R. prolixus* were fed as fourth (N4)- and fifth (N5)-instar nymphs, respectively.

One live specimen of each species was tested at each time point.

[a] Seven *T. dimidiata* and 8 *R. prolixus* nymphs were fed and tested; four *R. prolixus* specimens molted before testing.

[b] Nine *T. dimidiata* and *R. prolixus* nymphs were fed and all but one *R. prolixus* specimen survived to be tested; two *T. dimidiata* and four *R. prolixus* specimens molted before testing.

Table 3

Triatomine specimens by species and stage

Triatoma species and stage[a]	No.	*Trypanosoma cruzi* + /tested (%)	Bloodmeal ID + /tested (%)	Collection site[b]	County[c]
gerstaeckeri	70	40/57 (70)	33/70 (47)	dk,ih,oh,wn	B,C,G,U,Z
N1	2	0/2 (0)	0/2 (0)	wn	U
N2	5	3/5 (60)	5/5 (100)	wn	U
N3	l	0/l (0)	0/1 (0)	wn	U
N4	5	1/2 (50)	3/5 (60)	dk,wn	C,U
N5	7	1/3 (33)	2/7 (29)	dk,ih,wn	C,U,Z
Adult	50	35/44 (80)	23/50 (46)	dk,ih,oh	B,C,G,U,Z
indictiva	3	1/1 (100)	2/3 (67)	dk	C
Adult	3	1/1 (100)	2/3 (67)	dk	C
lecticularia	l	1/1 (100)	0/1 (0)	oh	U
Adult	l	1/1 (100)	0/1 (0)	oh	U
protracta	49	43/49 (88)	40/49 (82)	wn	U
N1	4	3/4 (75)	4/4 (100)	wn	U
N2	2	2/2 (100)	2/2 (100)	wn	U
N3	2	2/2 (100)	2/2 (100)	wn	U
N4	18	15/18 (83)	14/18 (78)	wn	U
N5	l9	17/19 (90)	17/19 (90)	wn	U
Adult	4	4/4 (100)	1/4 (25)	wn	U
sanguisuga	9	4/6 (67)	6/9 (67)	dk,ih,oh	B,C
Adult	9	4/6 (67)	6/9 (67)	dk,ih,oh	B,C
sanguisuga/indictiva	2l	not tested	15/21 (71)	dk	C
N5	2l	not tested	15/21 (71)	dk	C
Total	l53	89/114 (78)	96/153 (63)		

[a] N, nymph, no. refers to instar.

[b] dk, dog kennel; ih, inside house; oh, outside house; wn, woodrat nest.

[c] B, Bexar; C, Comal; G, Gonzales; U, Uvalde; Z, Zavala.

Table 4

Triatomine specimens with DNA sequence bloodmeal identifications

Host identification	No.	*Trypanosoma cruzi* + /tested (%)	*Triatoma* species[a]					Collection site[b]	County[c]
			g	*i*	*p*	*s*[d]	*s/i*		
Bos taurus (cow)	5	3/4 (75)	5	0	0	0	0	dk	C,Z
Canis lupus familiaris (dog)	19	9/12 (75)	10	2	0	6	1	dk,ih,oh	B,C,U,Z
Cathartes aura (turkey vulture)	1	1/1 (100)	0	0	0	1	0	dk	C
Coragyps atratus (black vulture)	1	Not tested	1	0	0	0	0	oh	C
Felis catus (cat)	6	5/5 (100)	6	0	0	0	0	dk,ih,oh	C,G,U
Gryllus texensis/rubens (field cricket)	15	Not tested	1	0	0	0	14	dk	C
Homo sapiens (human)	1	0/1 (0)	1	0	0	0	0	ih	B
Neotoma micropus (woodrat)	47	39/47 (83)	7	0	40	0	0	wn	U
Procyon lotor (raccoon)	1	1/1 (100)	1	0	0	0	0	dk	C
Sus scrofa (pig)	1	1/1 (100)	1	0	0	0	0	dk	C

[a] *g, gerstaeckeri; i, indictiva; p, protracta; s, sanguisuga; s/i, sanguisuga* or *indictiva*.

[b] dk, dog kennel; ih, inside house; oh, outside house; wn, woodrat nest.

[c] B, Bexar; C, Comal; G, Gonzales; U, Uvalde; Z, Zavala.

[d] One specimen was positive for both dog and turkey vulture DNA.