Biogeography and *Trypanosoma cruzi* Infection Prevalence of Chagas Disease Vectors in Texas, USA

Sonia A. Kjos,1* Karen F. Snowden,2 and Jimmy K. Olson1

Abstract

Data were pooled from multiple sources including newly collected triatomine specimens, preserved specimens, government reports, and scientific articles to create a biogeographical profile of triatomine vector species found in Texas. Triatomine specimens were documented in 97 of 254 counties, and *Trypanosoma cruzi*-infected specimens were reported from 48 counties. Triatomine specimens were distributed in 11 of the 12 ecoregions in Texas, with all but one species found in multiple ecoregions. Of the 241 newly collected specimens, 50.74% were infected with *T. cruzi*. *Triatoma gerstaeckeri* was the most frequently collected and most geographically dispersed species followed by *T. sanguisuga*. Three species, *T. gerstaeckeri*, *T. sanguisuga*, and *T. lecticularia*, were associated with human dwellings, and over half of the new specimens found inside or near houses were infected with *T. cruzi*. Chagas disease vectors in Texas are widely distributed and have adapted to ecologically diverse settings. The high *T. cruzi* infection prevalence of specimens found in close proximity to human settings suggests the presence of an active peridomestic Chagas disease transmission cycle.

Key Words: Triatoma—Vector—Chagas disease—Trypanosoma cruzi—GIS—North America.

Introduction

HAGAS DISEASE, CAUSED BY THE hemoflagellate protozoan parasite, Trypanosoma cruzi, continues to be an important public health threat in the western hemisphere with a prevalence of approximately 12 million human cases and 21,000 deaths annually (Schmunis 1999). Although transmission of the parasite to humans can occur by blood transfusion, organ donation, ingestion, or transplacentally, greater than 80% of all human cases are caused by vectorborne transmission (Dias and Schofield 1999). Members of the Triatominae (Hemiptera: Reduviidae) subfamily, which are obligate blood feeders in all postegg stages, are the biological arthropod vectors of T. cruzi. Transmission occurs when the infected bug defecates on or near the host during or shortly after feeding and the fecal material is subsequently rubbed into the bite wound, broken skin, or mucosal tissue. The lack of a vaccine and limited treatment options have kept the focus of Chagas disease prevention on control of the triatomine vectors (Dias and Schofield 1999, WHO 2002).

T. cruzi and its triatomine bug vectors have been discovered in diverse ecological niches throughout the tropical and subtropical regions of North and South America, approximately between the latitudes of 42°N and 46°S (Ramsey and

Schofield 2003). Of the 130 species currently recognized in the subfamily Triatominae (Schofield 2000), 11 have been reported in the southern half of the United States, eight in the state of Texas (Lent and Wygodzinski 1979, Ikenga and Richardson 1981). Triatomine bugs utilize a broad range of vertebrate hosts including mammals, birds, and reptiles, and all species are considered potential vectors of *T. cruzi* (Schofield 1994).

Molecular and morphological data suggest that the T. cruzi disease cycle, including the parasite, bug vectors, and susceptible mammalian hosts, became established in the United States long before the arrival of humans (Briones et al. 1999, Schofield 1988). The data on prevalence of T. cruzi in U.S. vectors are primarily derived from specimens collected in Texas, California, and Arizona with rates ranging from 17-48%, 14-40%, and 7.1-20.5%, respectively (Burkholder et al. 1980, Pippin 1970, Ryckman and Ryckman 1967, Sullivan et al. 1949, Wood 1949, 1975, Wood and Wood 1964). T. cruziinfected vectors have also been found in Florida (Beard et al. 1988), Georgia (Pung et al. 1995), Alabama (Olsen et al. 1964), Tennessee (Herwaldt et al. 2000), and Louisiana (Yaeger 1961). Although the reported incidence of human Chagas disease due to vector exposure in the United States is low, U.S. strains of T. cruzi have proven to be virulent as con-

¹Department of Entomology, Texas A&M University, College Station, Texas.

²Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas.

^{*}Present affiliation: CDC, Atlanta, Georgia.

firmed by clinical disease and death in a wide range of mammalian species including domestic dogs (Williams et al. 1977, Meurs et al. 1998), primates (Kasa et al. 1977, Gleiser et al. 1986), and humans (Packchanian 1943, Ochs et al. 1996).

In the past decade, geospatial methods have been increasingly applied to the study of the Chagas disease transmission cycle. Computerized vector distribution maps, vector ecological niche modeling, and disease transmission risk modeling have been used in Latin America to aid in Chagas disease vector control and disease prevention efforts (Beard et al. 2003, Costa et al. 2002, Dumonteil and Gourbiere 2004, Peterson et al. 2002). Conversely, little work has been done to comprehensively characterize the geospatial attributes of U.S. triatomine species. Usinger (1944) and Lent and Wygodzinsky (1979) rarely provide U.S. species distribution data below the state level in their widely cited works on Triatominae. Prior to 1970, several notations on field collections of U.S. triatomine species and corresponding T. cruzi infection rates were reported (Eads et al. 1963, Ryckman and Ryckman 1967, Sullivan et al. 1949, Wood 1941a, 1941b, 1941c), but these surveys were focused in small regions of the southwestern states. In the past three decades, little additional data have been reported to allow for accurate estimates of current triatomine distribution and infection prevalence in the United States. The goal of the present study was thus to assemble data on triatomine bugs regarding species identification, collection site attributes, and T. cruzi infection status from diverse sources to provide a comprehensive geospatial description of endemic vector species in Texas. Data were generated through new field studies, evaluation of preserved triatomine specimens, analysis of government reports, and abstraction of peer-reviewed journal articles. The state of Texas provided several advantages as the study area for this project. In addition to the availability of significant historical data on triatomine collections in the state, its geopolitical boundaries encompass a large land area (678,054 km²) and great ecological diversity. According to the U.S. Geological Survey, 12 major and 56 minor ecoregions are recognized within its borders (Griffith et al. 2004). Texas is located at the intersection of the distributions of eastern and western U.S. triatomine species and supports one of the highest triatomine species diversity in the U.S. (Lent and Wygodzinski 1979, Usinger 1944).

Materials and Methods

Collection of triatomine data

New triatomine specimens were collected from June 2005 to October 2006 from various sites within the state of Texas by an author (S.A.K.), employees of the Texas Department of State Health Services (TX DSHS), and Texas residents. Sites sampled by the author were selected on the basis of previous reports of triatomine bug sightings or canine Chagas disease cases. Specimens collected by health department employees and residents were primarily taken from in and around houses and dog kennels as prompted by concerns of disease transmission risk. All adult specimens were identified to the species level using the taxonomic key of Lent and Wygodzinsky (1979).

Preserved triatomine specimens from four Texas institutions (Midwestern State University, Wichita Falls; Texas A&M University, College Station; University of Texas Brack-

TABLE 1. SPECIMEN SOURCES USED IN A GEOSPATIAL STUDY OF *TRIATOMA* SPP. IN TEXAS, 2005–2006

Source	No.
Field collections	241
Preserved specimens	371
TX DSHS* records	123
Publications	1722
Total	2457

*Texas Department of State Health Services.

enridge Field Laboratory, Austin; and Wild Basin Wilderness Preserve, Austin) dating as far back as 1928 were reviewed for species identification and locality information. Species determinations were provided or verified for all preserved specimens. Finally, records of previously collected specimens were obtained from internal TX DSHS reports from 2002–2004 and from published peer-reviewed journal articles for the period 1941 to 2003 (Beard et al. 2003, Eads et al. 1963, Pippin 1970, Ryckman and Ryckman 1967, Sullivan et al. 1949, Williams et al. 1977, Wood 1941b). Data on collection location, species identification, and *T. cruzi* testing were abstracted from these records and combined with data from new and preserved specimens in a spreadsheet for analysis and mapping.

Detection of T. cruzi in new specimens

The posterior mid- and hindgut from each newly collected bug was dissected following previously described methods (Garcia and De Azambuja 1997) using sterile, disposable scalpels and forceps disinfected with a 10% bleach solution and ultrapure water rinse between samples. The excised gut was placed in 50- μ L of molecular-grade water and homogenized using a sterile, disposable pestle. A 5- μ L aliquot of homogenate was examined by direct microscopy using Nomarski optics at 400× for the presence of trypanosomes. The remaining sample was stored at -70° C for DNA analysis. DNA was extracted from all gut samples using the PURE-GENE[®] DNA Purification Kit (Gentra, Minneapolis, MN) and used as template for polymerase chain reaction (PCR) amplification.

One of two primer sets was used for detection of T. cruzi in all samples: Tc24T1F-Tc24T2R (Vera-Cruz et al. 2003), which target a ~550-bp segment of a T. cruzi-specific flagellar protein gene, and 609F (Da Silva et al. 2004) and TcV5R (5'-ACT CTT GCG AAC GTA CTC CCC-3'), which target a ~900-bp DNA segment containing small subunit (SSU) rRNA sequence. Use of the second primer set was to facilitate data collection for a phylogenetic analysis of the *T. cruzi* isolates, the results of which will be reported separately. Amplifications were done in $25-\mu$ L reactions containing $1-\mu$ L of DNA template, $1 \times$ GoTaq Green Master Mix (Promega, Madison, WI), MgCl₂ final concentration adjusted to 2.5 mM, and 10 pM of primers. After an initial denaturation at 95°C for 1 minute, the reactions were cycled 35 times for 45 seconds at 95°C, 45 seconds at 58°C, and 90 seconds at 72°C, followed by a final extension for 7 minutes at 72°C. The amplified products were analyzed on 1.5% agarose gels with ethidium bromide. No further processing was performed on samples tested with the Tc24T1F-Tc24T2R primers due to the



FIG. 1. Distribution of *Triatoma* spp. and *T. cruzi*-infected bugs in Texas by county 1928–2006.

demonstrated specificity of this primer set for T. cruzi. Amplified SSU rRNA gene products positive for a band at ~900 bp were extracted from a low melt 1.5% agarose gel and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). The purified amplification products were sequenced using 2.5 μ L of Perkin Elmer ABI Big Dye Reaction Mix plus 10 pM of primer in a PCR. The reactions were cycled 45 times for 10 seconds at 95°C, 5 seconds at 50°C, and 4 minutes at 60°C, followed by a holding temperature of 4°C. The products were analyzed on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) by the Gene Technologies Laboratory at Texas A&M University. Consensus sequences were constructed and edited using Sequencher software (Gene Codes Corp., Ann Arbor, MI) and compared to sequences in GenBank using the BLASTN algorithm (McGinnis and Madden 2004) for trypanosome species identification.

Geospatial analysis

The geographic locations for new specimens were obtained by a mobile GPS unit or geocoded from a street address. Geographic locations for all other specimens were abstracted from locality labels (preserved specimens) or specimen records (TX DSHS and published articles) and geocoded. County-level location data for all specimens and decimal degree (i.e., point) locations for specimens with specific collection site information were mapped using ArcGIS 9.1 software (ESRI, Redlands, CA). Triatomine species distribution by ecoregion was obtained by combining specimen point locations and the Texas ecoregions map, level 3 categories (Griffith et al. 2004).

Results

Geographic distribution of triatomine bugs

The distribution of triatomine bugs in Texas was derived from 2457 records. This number includes multiple specimens collected from the same location in some cases. Table 1 provides the distribution of triatomine specimen records by source. The accumulated data included records on each of seven *Triatoma* species previously reported from Texas: *T. gerstaeckeri* (Stål), *T. indictiva* Neiva, *T. lecticularia* (Stål), *T. neotomae* Neiva, *T. protracta* (Uhler), *T. rubida* (Uhler), and *T. sanguisuga* (Leconte).



FIG. 2. *Triatoma* species density map and individual species maps by county in Texas, 1928–2006. Black dots represent new collections from 2005–2006. Photographs of specimens are not displayed in relative scale to one another.

Based on data analyzed in this study, the triatomine bug distribution in Texas covers 97 of 254 counties. Of the 67 counties with data on *T. cruzi* testing in triatomine specimens, 48 had at least one positive result. Figure 1 shows the distribution of all triatomine specimens and *T. cruzi*-infected triatomine specimens by county. The distribution of triatomine bugs in Texas is widespread, encompassing all major geographic regions. The distribution of *T. cruzi*-infected bugs includes most geographic regions with the exception of the northern third of the state.

Triatoma species density and individual species maps by county are provided in Figure 2. The collection sites of new triatomine specimens are also indicated in Figure 2. The greatest species diversity was found primarily in the mid to south-central portion of the state. Seven counties (Cameron, Dimmit, Duval, Hidalgo, Travis, Uvalde, and Webb) accounted for the highest number of species, with four each. Of the seven species, *T. gerstaeckeri* had the broadest distribution, covering all but the northern expanse of Texas. *T. sanguisuga* and *T. lecticularia* were nearly sympatric in their

distributions, covering all but the far west and panhandle regions. *T. protracta* and *T. indictiva* locations extended from west to the north and south-central regions. *T. rubida* and *T. neotomae* had the most confined distributions within the state. *T. rubida* was found exclusively in the far west region of the state. *T. neotomae* was restricted to the mid and deep southcentral regions.

Distribution by ecoregion was determined for all triatomine bugs having recorded point locations (623 of 2457 records). Each ecoregion represents a distinct area defined by similarity in ecosystems and environmental resources (Griffith et al. 2004). Triatomine species were found in 11 of the 12 level 3 ecoregions of Texas. The number of unique point locations for each of the species used in the ecoregion analysis was as follows: *T. gerstaeckeri*, 114; *T. sanguisuga*, 51; *T. lecticularia*, 25; *T. protracta*, 24; *T. indictiva*, 11; *T. rubida*, 9; and, *T. neotomae*, 2.

The greatest species diversity was found in the southern Texas plains with five species and the Chihuahuan Deserts, Cross Timbers, Edwards Plateau, and Western Gulf coastal

Ecoregion	Gerstaeckeri	Lecticularia	Sanguisuga	Indictiva	Protracta	Rubida	Neotomae
Southern Texas Plains	26.8	16.0	9.8	9.1	29.2		
Chihuahuan Deserts	8.0	_	_	9.1	45.8	88.9	
Cross Timbers	0.9	20.0	9.8		4.2	_	
Edwards Plateau	22.3	4.0	9.8	63.6			
Western Gulf Coastal Plain	32.1	16.0	19.6				100.0
East Central Texas Plains	3.6	8.0	25.5				
High Plains	_	_	_	9.1	12.5	11.1	_
Texas Blackland Prairies	4.5	32.0	17.6		_		
Central Great Plains	1.8	_	_	9.1			
South Central Plains	_	4.0	7.8		_		_
Southwestern Tablelands	_	_	_		8.3		
Arizona New Mexico Mtns	_	_	_		_		
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

TABLE 2. TRIATOMA SPECIES DISTRIBUTION BY TEXAS ECOREGIONS (PERCENT)

plains each with four species. Most species occurred in more than one ecoregion, with the exception of *T. neotomae*, which was found solely in the Western Gulf coastal plains region. *T. gerstaeckeri* was found in the greatest number of ecoregions, with eight, followed by *T. lecticularia* and *T. sanguisuga*, with seven each. Table 2 provides a list of triatomine species by ecoregion.

Characterization of new specimens

A total of 241 triatomine specimens was collected from 34 counties at 57 unique locations in Texas. Of the specimens collected, 233 were adults and eight were immatures (fourth and fifth instar nymphs). At least one specimen from seven triatomine species previously reported in the state was collected. *T. gerstaeckeri* was collected most frequently with 180 specimens followed by *T. sanguisuga* with 38. At least one specimen from five of the seven species collected tested positive for *T. cruzi* (*T. gerstaeckeri*, *T. indictiva*, *T. lecticularia*, *T. protracta*, and *T. sanguisuga*). The new specimens, categorized by species and *T. cruzi* testing results, are shown in Table 3.

Of the 241 specimens collected, 203 were tested for the presence of *T. cruzi*. The infection rate for tested specimens was 50.74% (103/203). Of the adult specimens tested, 52% were positive (102/197), and of the immature specimens tested, 17% were positive (1/6). With the exception of one specimen, all bugs that tested positive for trypanosomes by microscopy also tested positive by PCR. It is likely that the

DNA from the one microscopy-positive/PCR-negative sample was lost or degraded during the extraction process or was below detection sensitivity. For the purposes of the current study, this specimen was treated as negative for *T. cruzi*, because the species of trypanosome observed by microscopy could not be determined. Another morphologically similar trypanosome, *Blastocrithidia* spp., was identified in three of the specimens in this study (Kjos et al. 2008a). Of the 103 PCR-positive specimens, 21 were negative by microscopy. All but one of the 21 specimens were dead prior to dissection. Samples from 31 specimens were not tested by microscopy due to prior storage by freezing or alcohol, rendering direct microscopy ineffective for trypanosome detection.

The majority (68%) of new specimens were collected from domestic settings (i.e., inside or near houses, or associated with bite events), followed by dog kennels (18%), and sylvatic settings (14%), which were primarily woodrat (*Neotoma micropus*) nests (Table 4). Of bug specimens tested for *T. cruzi* infection, 54% were positive among those collected from domestic settings and dog kennels, and 30% were positive among those found in sylvatic settings. Of the eight immature specimens (nymphs) collected, two were collected from inside houses, four from outside surfaces of houses, one from a dog kennel, and one from a woodrat nest. Only the nymph from the dog kennel tested positive for *T. cruzi*. Multiple *Triatoma* species were collected from 8 of the 57 sites: domestic settings (six sites), dog kennels (one site), and sylvatic settings (one site).

TABLE 3.FIELD-COLLECTED TRIATOMINE SPECIMENS IN TEXAS,2005–2006, BY SPECIES AND T. CRUZI INFECTION STATUS

Triatoma species	T. cruzi positive	T. cruzi negative	Not tested	Total
Gerstaeckeri	86	70	24	180
Sanguisuga	10	19	9	38
Lecticularia	3	2	3	8
Unknown (nymphs)	1	5	2	8
Indictiva	1	1	0	2
Neotomae	0	2	0	2
Protracta	2	0	0	2
Rubida	0	1	0	1
Total	103	100	38	241

	Inside house	Human bite	Near house	Dog kennel	Sylvatic	Total
<i>T. cruzi</i> positive (%)*	15 (47)	6 (86) 1	52 (54)	22 (56)	8 (30)	103 (51)
Not tested	4	$0 \\ 7$	24 120	4	6 33	36 241

TABLE 4.FIELD-COLLECTED TRIATOMINE SPECIMENS IN TEXAS,2005–2006, by Site and T. cruzi Infection Status

*Of tested specimens.

Discussion

This study provides new information on the distribution and infection prevalence of triatomine species in Texas. The last extensive survey of Texas triatomine species was based on field collections primarily from sylvatic settings in 40 counties during the period 1941 to 1947 (Sullivan et al. 1949). The current study expanded on these results to include data from an additional 57 counties, providing more comprehensive information on the distribution of triatomine species in Texas. The new specimens collected during this study provide confirmation of species distributions determined from a large portion of preserved specimens and published data (Fig. 2). Similar to the 1949 survey, T. gerstaeckeri was the most commonly collected species followed by T. sanguisuga. In areas of Texas where there have not been reports of either T. cruzi-infected triatomine bugs or triatomine bugs at all, we were not able to determine if it was due to a lack of data or a true absence. Although systematic sampling and testing of bugs in these areas of the state would have been informative in this regard, it was beyond the scope of this study. The ecological characteristics of these areas are similar to areas with known occurrences of triatomine bugs, so it is likely that the bugs exist there as well.

As described previously, eight species have been reported from the state of Texas. We could find only a single Texas collection report of one of these species, *T. recurva*, found during field studies in Brewster County, Texas (Ikenga and Richerson 1984). Aside from this one account, this species has only been reported from Arizona and northwestern Mexico (Pfeiler et al. 2006, Usinger 1944, Wood 1941b, 1949). We obtained data on 21 triatomine specimens collected from Brewster County in 12 different years from 1928 to 1995. The species collected included *T. gerstaeckeri* (10), *T. protracta* (9), and *T. rubida* (3). No data on collections of *T. recurva* in Texas were found other than the account by Ikenga et al. Additional field studies would be informative to confirm whether or not this area of Texas represents a new distribution for this species.

There is considerable geographic overlap in species distribution. No single species appears to occupy an exclusive region within Texas. *T. gerstaeckeri*, *T. sanguisuga*, *T. indictiva*, *T. lecticularia*, and *T. protracta* all share a large area encompassing the north, mid, and south-central regions. Not surprisingly, these same species are spread across diverse ecological areas within Texas, with each of these species occupying a minimum of five ecoregions. *T. rubida* and *T. neotomae* appear to be more restricted in habitat type, occupying only two and one ecoregions, respectively. However,

the small number of point locations used in the ecoregions analysis of these two species may limit the interpretation of these findings. Analysis of the species using county-level data (as opposed to point locations) shows that T. neotomae has also been found in an area encompassed by the Edwards Plateau ecoregion, which would expand the distribution of this species to two ecoregions. Although U.S. species have been associated with a broad range of hosts, they are frequently found in woodrat nests in the western half of the country. Little has been reported on the natural history of *T*. *neotomae*, other than that it has been almost exclusively collected from woodrat nests (Lent and Wygodzinski 1979). Of the new adult specimens collected in this study, several T. gerstaeckeri and T. lecticularia specimens were found in woodrat nests, and both specimens of T. neotomae were found in dog kennels located in close proximity to woodrat nests.

The overall *T. cruzi* infection rate for new triatomine specimens in this study (50.74%) was higher than previous reports of 17–48% (Burkholder et al. 1980, Pippin 1970, Sullivan et al. 1949). The seven species analyzed in this study have previously been found to be naturally infected with *T. cruzi* in Texas (Pippin et al. 1968, Sullivan et al. 1949). Data generated from new specimens collected during this study support a single earlier account of *T. indictiva* found naturally infected with *T. cruzi* (Pippin et al. 1968).

PCR analysis of gut samples proved to be more sensitive than direct microscopy in the analysis of new specimens in this study. Of the 21 samples that were microscopy-negative/PCR-positive, 20 were extracted from dead bug specimens, suggesting that direct microscopy is less sensitive when analyzing dead specimens. When analyzing the live specimens, only one positive specimen was missed by direct microscopy. Direct microscopy proved to be less specific than PCR analysis of gut material in this study. Several samples with *T. cruzi*-like trypanosomes as observed by microscopy were either infected with another species of trypanosome or had dual infections (*T. cruzi* and *B. triatomae*) as determined by PCR and DNA sequence analysis (Kjos et al. 2008a).

Of the new triatomine specimens collected from dog kennels, over half of those tested were positive for *T. cruzi*. In a related study on the prevalence of Chagas disease in canines, 537 serologically or histopathologically confirmed cases were identified over a 15-year period in domestic dogs residing in Texas (Kjos et al. 2008b). The presence of both infected dogs and infected vectors in close proximity to human residences in Texas provides evidence for the presence of an active peridomestic Chagas disease transmission cycle.

A high percentage of triatomine specimens found in close association with humans in this study was infected with *T*.

cruzi. Of bugs collected from domestic settings (inside or near houses), 53% of those tested were infected, including six of seven that were associated with human bites. T. gerstaeckeri, T. sanguisuga, and T. lecticularia were identified among the specimens found inside or near houses, and T. gerstaeckeri and *T. sanguisuga* were associated with human bites. Of the 180 T. gerstaeckeri specimens collected, 113 (63%) were found in domestic settings. This particular species has been frequently associated with human dwellings. In a survey of villages in northeastern Mexico, where T. gerstaeckeri is considered an important human vector, 181 of 192 (94%) specimens were found in or around houses (Martinez-Ibarra et al. 1992). Data from our study support previously published reports on prevalence of human triatomine bites in the United States, indicating that close association between humans and triatomine bugs does occur (Frazier 1974, Lane et al. 1997, Lynch and Pinnas 1978, Moffitt et al. 2003). Primarily adult stages of the bugs were collected in domestic settings according to data analyzed in this study, suggesting that colonization of houses by U.S. species (as evidenced by finding immature stages) continues to be a rare event.

Accounts of triatomine bugs encountered inside houses in the United States have been sporadically reported for the past 60 years (Beard et al. 1988, Griffith 1948, Navin et al. 1985, Wood and Wood 1964), but few human Chagas disease cases due to vector exposure have been reported. The first autochthonous U.S. case was reported in 1955 (Woody and Woody 1955), and five other cases have been reported since (Dorn et al. 2007, Herwaldt et al. 2000, Ochs et al. 1996, Schiffler et al. 1984, TDH 1956). Three of the cases were in Texas residents. One additional case from Texas was identified in 2006 involving a small child residing in the southern region of the state (L. Robinson, Texas Department of State Health Services, personal communication). Twelve triatomine bug specimens were collected by Texas Department of State Health Services personnel from the residence of this case and submitted to one of us (S.A.K.) for identification and testing. All were adult T. gerstaeckeri and one of two specimens tested was positive for T. cruzi. The remaining specimens were not tested due to their deteriorated state.

It may be appropriate to extrapolate results from the data gathered on biogeography of Chagas disease vectors in Texas to adjacent U.S. states and Mexico where landscape features and ecoregions are similar. All seven species found in Texas have distributions that extend beyond the geopolitical boundaries of the state. Chagas disease vectors are ubiquitous across Texas and have adapted to a wide range of ecological settings including habitats near human residences. *T. cruzi* infection prevalence is high among triatomine bugs, and infected bugs have been found in most regions of the state. The factors influencing the risk of vector-borne transmission of Chagas disease in the United States, such as frequency and type of exposure to humans, vector host preference and host range, and distribution of virulent parasite strains, require further analysis.

Acknowledgments

We thank the many Texas Department of State Health Services and Texas A&M University personnel who provided data or specimens, especially Roy Burton, Dr. Eric Fonken, Richard Klein, Dr. Forrest Mitchell, Dr. Beverlee Nix, Greg Pye, Dr. Laura Robinson, Jim Schuermann, Robyn Seiferth, Aaron Smith, Glenna Teltow, Dr. Noel Troxclair, Dr. Catherine Tull, and Katherine von Alt, as well as the many Texas residents that submitted specimens. We also acknowledge Dr. Joe Gillespie for help with primer design, Kathleen Logan for assistance with optimization of molecular assays, and the curators of the institution collections used in the study: Ed Riley, Texas A&M University; Dr. John Abbott, University of Texas; Dr. Roy Vogtsberger, Midwestern State University; and Kiki Corry, Wild Basin Wilderness Preserve. We would also like to acknowledge the anonymous reviewers whose careful critique improved the quality of the manuscript.

Disclosure Statement

Funding for this study was provided to S.A.K. by the Texas Mosquito Control Association, James Gus Foyle Memorial Scholarship.

References

- Beard, CB, Pye, G, Steurer, FJ, Rodriguez, R, et al. Chagas disease in a domestic transmission cycle, southern Texas, USA. Emerg Infect Dis 2003; 9:103–105.
- Beard, CB, Young, DG, Butler, JF, Evans, DA. First isolation of *Trypanosoma cruzi* from a wild-caught *Triatoma sanguisuga* (LeConte) (Hemiptera: Triatominae) in Florida, U.S.A. J Parasitol 1988; 74:343–344.
- Briones, MR, Souto, RP, Stolf, BS, Zingales, B. The evolution of two *Trypanosoma cruzi* 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.
- 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.
- Costa, J, Peterson, AT, Beard, CB. Ecologic niche modeling and differentiation of populations of *Triatoma brasiliensis* Neiva, 1911, the most important Chagas' disease vector in northeastern Brazil (hemiptera, reduviidae, triatominae). Am J Trop Med Hyg 2002; 67:516–520.
- Da Silva, FM, Noyes, H, Campaner, M, Junqueira, AC, et al. Phylogeny, taxonomy and grouping of *Trypanosoma rangeli* isolates from man, triatomines and sylvatic mammals from widespread geographical origin based on SSU and ITS ribosomal sequences. Parasitology 2004; 129:549–561.
- Dias, J, Schofield, C. The evolution of Chagas disease (American trypanosomiasis) control after 90 years since Carlos Chagas discovery. Mem Inst Oswaldo Cruz 1999; 94(Suppl 1):103– 121.
- Dorn, PL, Perniciaro, L, Yabsley, MJ, Roellig, DM, et al. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emerg Infect Dis 2007; 13:605–607.
- Dumonteil, E, Gourbiere, S. Predicting *Triatoma dimidiata* abundance and infection rate: a risk map for natural transmission of Chagas disease in the Yucatan peninsula of Mexico. Am J Trop Med Hyg 2004; 70:514–519.
- Eads, RB, Trevino, HA, Campos, EG. *Triatoma* (Hemiptera: Reduviidae) infected with *Trypanosoma cruzi* in south Texas wood rat dens. Southwestern Naturalist 1963; 8:38–42.
- Frazier, CA. Biting insect survey: a statistical report. Ann Allergy 1974; 32:200–204.

- Garcia, ES, De Azambuja, P. Infection of triatomines with *Try*panosoma cruzi. In: Crampton, JM, Beard, CB, and Louis, C., eds. *Molecular biology of disease vectors*. New York, Chapman & Hall, 1997:146–155.
- Gleiser, CA, Yaeger, RG, Ghidoni, JJ. *Trypanosoma cruzi* infection in a colony-born baboon. J Am Vet Med Assoc 1986; 189:1225–1226.
- Griffith, GE, Bryce, SA, Omernik, JM, Comstock, JA, et al. Ecoregions of Texas (color poster with map, descriptive text, and photographs). Reston, Virginia: U.S. Geological Survey, 2004. Available at: www.lib.utexas.edu/maps/texas.html. Last accessed December 7, 2007.
- Griffith, ME. The bloodsucking conenose, or "big bedbug," *Triatoma sanguisuga* (Leconte), in an Oklahoma City household. Proceedings of the Oklahoma Academy of Science 1948; 28:24–27.
- Herwaldt, BL, Grijalva, MJ, Newsome, AL, McGhee, CR, 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.
- 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.
- Kasa, TJ, Lathrop, GD, Dupuy, HJ, Bonney, CH, et al. An endemic focus of *Trypanosoma cruzi* infection in a subhuman primate research colony. J Am Vet Med Assoc 1977; 171:850–854.
- Kjos, SA, Gillespie, JJ, Olson, JK, Snowden, KF. Detection of *Blastocrithidia* spp. (Kinetoplastida: Trypanosomatidae) in Chagas Disease Vectors from Texas, USA. Vector Borne Zoonotic Dis DOI: 10.1089/vbz.2008.0027.
- Kjos, SA, Snowden, KF, Craig, TM, Lewis, B, et al. Distribution and characterization of canine Chagas Disease in Texas. Vet Parasitol 2008b; 152:249–256.
- Lane, JE, Olivares-Villagomez, D, Vnencak-Jones, CL, McCurley, TL, et al. Detection of *Trypanosoma cruzi* with the polymerase chain reaction and *in situ* hybridization in infected murine cardiac tissue. Am J Trop Med Hyg 1997; 56:588–595.
- Lent, H, Wygodzinski, P. Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas disease. Bull Am Mus Nat Hist 1979; 163:123–520.
- Lynch, PJ, Pinnas, JL. "Kissing bug" bites. *Triatoma* species as an important cause of insect bites in the southwest. Cutis 1978; 22:585–591.
- Martinez-Ibarra, JA, Galaviz-Silva, L, Campos, CL, Trujillo-Garcia, JC. Distribucion de los triatominos asociados al domicilio humano en el municipio de general Teran, Nuevo Leon, Mexico. Southwestern Entomologist 1992; 17:261–264.
- McGinnis, S, Madden, TL. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res 2004; 32:W20–W25.
- Meurs, KM, Anthony, MA, Slater, M, Miller, MW. Chronic *Try*panosoma cruzi infection in dogs: 11 cases (1987–1996). J Am Vet Med Assoc 1998; 213:497–500.
- Moffitt, JE, Venarske, D, Goddard, J, Yates, AB, et al. Allergic reactions to *Triatoma* bites. Ann Allergy Asthma Immunol 2003; 91:122–128; quiz 128–130, 194.
- Navin, TR, Roberto, RR, Juranek, DD, Limpakarnjanarat, K, et al. Human and sylvatic *Trypanosoma cruzi* infection in California. Am J Public Health 1985; 75:366–369.
- Ochs, DE, Hnilica, VS, Moser, DR, Smith, JH, 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.

- Olsen, PF, Shoemaker, JP, Turner, HF, Hays, KL. Incidence of *Trypanosoma cruzi* (Chagas) in wild vectors and reservoirs in East-Central Alabama. J Parasitol 1964; 50:599–603.
- Packchanian, A. The infectivity of the Texas strain of *Trypansoma* cruzi to man. Am J Trop Med 1943; 23:309–314.
- Peterson, AT, Sanchez-Cordero, V, Beard, CB, Ramsey, JM. Ecologic niche modeling and potential reservoirs for Chagas disease, Mexico. Emerg Infect Dis 2002; 8:662–667.
- Pfeiler, E, Bitler, BG, Ramsey, JM, Palacios-Cardiel, C, et al. Genetic variation, population structure, and phylogenetic relationships of *Triatoma rubida* and *T. recurva* (Hemiptera: Reduviidae: Triatominae) from the Sonoran Desert, insect vectors of the Chagas' disease parasite *Trypanosoma cruzi*. Mol Phylogenet Evol 2006; 41:209–221.
- Pippin, WF. The biology and vector capability of *Triatoma san-guisuga texana* Usinger and *Triatoma gerstaeckeri* (Stal) compared with *Rhodnius prolixus* (Stal) (Hemiptera: Triatominae). J Med Entomol 1970; 7:30–45.
- Pippin, WF, Law, PF, Gaylor, MJ. Triatoma sanguisuga texana Usinger and Triatoma sanguisuga indictiva Neiva naturally infected with Trypanosoma cruzi Chagas in Texas (Hemiptera: Triatominae) (Kinetoplastida: Trypanosomidae). J Med Entomol 1968; 5:134.
- Pung, OJ, Banks, CW, Jones, DN, Krissinger, MW. *Trypanosoma cruzi* in wild raccoons, opossums, and triatomine bugs in southeast Georgia, U.S.A. J Parasitol 1995; 81:324–326.
- Ramsey, JM, Schofield, CJ. Control of Chagas disease vectors. Salud Publica Mex 2003; 45:123–128.
- Ryckman, RE, Ryckman, JV. Epizootiology of *Trypanosoma cruzi* in southwestern North America. XII. Does Gause's rule apply to the ectoparasitic Triatominae? (Hemiptera: Reduviidae) (Kinetoplastidae: Trypanosomidae) (Rodentia: Cricetidae). J Med Entomol 1967; 4:379–386.
- Schiffler, RJ, Mansur, GP, Navin, TR, Limpakarnjanarat, K. Indigenous Chagas' disease (American trypanosomiasis) in California. JAMA 1984; 251:2983–2984.
- Schmunis, GA. Iniciativa del Cono Sur. In: Schofield, CJ, Ponce, C, eds. Proceedings of the Second International Workshop on Population Biology and the Control of Triatominae. Mexico City, IN-DRE, 1999:26–31.
- Schofield, CJ. Biosystematics of the Triatominae. In: Service, MW, ed. Systematics association special volume. Oxford, Clarendon Press, 1988:284–312.
- Schofield, CJ. Biosystematics and evolution of the Triatominae. Cad Saude Publica 2000; 16(Suppl 2):89–92.
- Schofield, C. Triatominae biology & control. West Sussex, Eurocommunica Publications, 1994.
- Sullivan, TD, McGregor, T, Eads, RB, Davis, DJ. Incidence of *Try-panosoma cruzi*, Chagas, in *Triatoma* (Hemiptera: Reduviidae) in Texas. Am J Trop Med 1949; 29:453–458.
- TDH (Texas Department of Health). Found: Two cases of Chagas disease. Tex Health Bull 1956; 9:11–13.
- Usinger, RL. The Triatominae of North and Central America and the West Indies and their public health significance. Washington, D.C., U.S. Government Printing Office, 1944.
- Vera-Cruz, JM, Magallon-Gastelum, E, Grijalva, G, Rincon, AR, et al. Molecular diagnosis of Chagas' disease and use of an animal model to study parasite tropism. Parasitol Res 2003; 89:480–486.
- WHO (World Health Organization). Control of Chagas disease. World Health Organ Tech Rep Ser 2002; 905:i–vi, 1–109, back cover.
- Williams, GD, Adams, LG, Yaeger, RG, McGrath, RK, et al. Naturally occurring trypanosomiasis (Chagas' disease) in dogs. J Am Vet Med Assoc 1977; 171:171–177.

BIOGEOGRAPHY AND T. CRUZI PREVALENCE OF VECTORS IN TEXAS

- Wood, SF. New localities for *Trypanosoma cruzi* Chagas in southwestern United States. Am J Hyg 1941a; 34:1–13.
- Wood, SF. Notes on the distribution and habits of reduviid vectors of Chagas' disease in the southwestern United States. Part I. The Pan-Pacific Entomologist 1941b; 17:85–94.
- Wood, SF. Notes on the distribution and habits of reduviid vectors of Chagas' disease in the southwestern United States. Part II. The Pan-Pacific Entomologist 1941c; 17:115–118.
- Wood, SF. Additional observations on *Trypanosoma cruzi*, Chagas, from Arizona in insects, rodents, and experimentally infected animals. Am J Trop Med 1949; 29:43–55.
- Wood, SF. *Trypanosoma cruzi*: new foci of enzootic Chagas' disease in California. Exp Parasitol 1975; 38:153–160.
- Wood, SF, Wood, FD. Nocturnal aggregation and invasion of homes in southern California by insect vectors of Chagas' disease. J Econ Entomol 1964; 57:775–776.

- Woody, NC, Woody, HB. American Trypanosomiasis (Chagas' disease): first indigenous case in the United States. JAMA 1955; 159:676–677.
- Yaeger, RG. The present status of Chagas' disease in the United States. Bull Tulane Univ Med Fac 1961; 21:9–13.

Address reprint requests to: Dr. Sonia A. Kjos Centers for Disease Control and Prevention CCID/NCZVED/DPD/Entomology Branch MS F-42 4770 Buford Highway Chamblee, GA 30341

E-mail: skjos@cdc.gov