RESEARCH ARTICLE

Surveillance of *Trypanosoma cruzi* infection in Triatomine vectors, feral dogs and cats, and wild animals in and around El Paso county, Texas, and New Mexico

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Abstract

The causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by triatomine vectors. The insect is endemic in the Americas, including the United States, where epidemiological studies are limited, particularly in the Southwestern region. Here, we have determined the prevalence of *T. cruzi* in triatomines, feral cats and dogs, and wild animals, the infecting parasite genotypes and the mammalian host bloodmeal sources of the triatomines at four different geographical sites in the U.S.-Mexico border, including El Paso County, Texas, and nearby cities in New Mexico. Using qualitative polymerase chain reaction to detect *T. cruzi* infections, we found 66.4% (n = 225) of triatomines, 45.3% (n = 95) of feral dogs, 39.2% (n = 24) of feral cats, and 71.4% (n = 7) of wild animals positive for *T. cruzi*. Over 95% of *T. cruzi* genotypes or discrete typing units (DTUs) identified were TcI and some TcIV. Furthermore, *Triatoma rubida* was the triatomine species most frequently (98.2%) collected in all samples analyzed. These findings suggest a high prevalence of *T. cruzi* infections among triatomines, and feral and wild animals in the studied sites. Therefore, our results underscore the urgent need for implementation of a systematic epidemiological surveillance program for *T. cruzi* infections in insect vectors, and feral and wild animals, and Chagas disease in the human population in the southwestern region of the United States.

Author summary

Chagas disease is caused by the parasite *Trypanosoma cruzi* and one of the major transmission routes is the contaminated feces of blood-feeding triatomine insect vectors, popularly known as kissing bugs. In recent years, this disease has become an important public health concern to the United States and other nonendemic regions of the world. Despite many studies about the prevalence of *T. cruzi* in triatomines, and domestic, feral and wild animals in central and southern Texas, there have been no studies in west Texas and New Mexico. In this study, we report the presence of triatomines in residences in El Paso County, TX, and surrounding communities in New Mexico (cities of Anthony and Las Cruces), as well as *T. cruzi* infections in feral and wild animals. Using two molecular techniques to analyze the bloodmeal source in triatomines, we detected 12 different mammalian bloodmeal sources, including human and canine. Finally, parasite genotyping showed that most (95%) of the samples belonged to the genotype TcI, which is prevalent in North America. Our findings indicate that the El Paso County and surrounding communities (>950,000 people) are high risk areas for *T. cruzi* transmission to humans, feral cats and dogs, and wild animals. Thus, there is an urgent necessity for a public health epidemiological surveillance program for *T. cruzi* infections in kissing bugs, feral and wild animals, and in the human population in the U.S.-Mexico border region.

Introduction

The vector-borne parasite *Trypanosoma cruzi* causes Chagas disease. The parasite infects many mammals including humans, domestic and wild animals in the Americas [1]. An estimated 8 million people are infected in Latin America [2] and approximately 300,000 chronically infected people live in the United States [3]. The infection in humans and animals can be asymptomatic or symptomatic, progressing from an acute phase with flu-like symptoms to a chronic cardiac and/or gastrointestinal (GI) disease that can lead to heart failure, GI megasyndromes and/or sudden death [1,4,5]. There is no human or veterinarian vaccine, and chemotherapy options are of limited efficacy, and exhibit frequent adverse events and variable outcomes [6]. Transmission to mammals occurs after the introduction of infected triatomine fecal material into a wound or mucous membrane, as well as by the oral (consumption of foods and juices contaminated with *T. cruzi*-infected kissing bugs or their feces), congenital, and/or transfusion/transplantation routes [7]. The nocturnal triatomine vector, also known as "kissing bug", serves as the main mode of transmission, particular in established sylvatic and domestic transmission cycles [8]. Around 100 different wildlife mammalian species are competent reservoirs of *T. cruzi* [9] and at least 24 species have been recognized as natural wildlife reservoirs in the United States [2], with canines being the most important component of peridomestic transmission, forming a connection between sylvatic and domestic transmission cycles [10,11]. Lastly, human infections can occur when triatomines establish nests near houses and triatomines feed on both humans and animals [12].

There are 141 currently recognized triatomines species in the Americas, many of which can be infected by and transmit *T. cruzi* [13]. Of those, 11 species are native to the United States, distributed across the southern half of the country from East to West [2]. Seven of these species have been collected in Texas and all have the potential to transmit *T. cruzi* [14]. Although triatomines are an important source of human infections, canines are more likely to be infected with *T. cruzi* than humans because of behavioral factors, such as the ingestion of the triatomines by dogs that cause infection through the oral route and because canines commonly

sleep outside, increasing the chances of the vector feeding on them [9,15–17]. In Texas, the transmission cycle includes seven reported triatomine species and 27 wild mammalian reservoirs, with many more mammalian reservoirs potentially involved in the cycle [18]. Finally, some human studies in Texas have reported autochthonous transmission of *T. cruzi* to humans [19–23].

While there is increasing evidence of Chagas disease in Texas, epidemiologic assessment studies on this disease have not been conducted in southwest Texas, which includes El Paso, an urban border city with a population of over 830,000 [24]. Therefore, in this study, we evaluated the prevalence of *T. cruzi* in wild triatomines, and peridomestic stray or feral dogs and cats, and wild animals in El Paso and surrounding communities. All *T. cruzi* positive samples were further characterized to determine the genotype or discrete typing units (DTU) of the parasite, an important genetic marker, as well as the mammalian source of the bloodmeals for triatomines. An understanding of the prevalence, distribution, and genetic profile of *T. cruzi* in triatomines, and feral and wild animals provided an estimate of the disease risk in both peridomestic and rural settings. Bloodmeal analysis provided critical information on the components of transmission cycles in El Paso area and surrounding areas, as well as transmissibility risks.

Methods

Ethics statement

The blood and tissue samples from peridomestic, feral dogs and cats, and wild animals were collected by the veterinarian of the Texas Department of State Health Services, Zoonosis Control Region 10 in the El Paso and surrounding urban and rural communities. The samples from dogs and cats were obtained from a local animal shelter and coordinates of capture location were provided according to the approved protocols A-201408-1, and by the UTEP's Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) protocol numbers 2014–04, 1608423, and 1111061–1.

Triatomine collection, identification, and DNA extraction

Triatomine insects were collected from May 2016 to September 2019 (between May and September of each year) in peridomestic and rural areas in El Paso County and Indio Mountains Research Station (IMRS) in Hudspeth, Texas. Collections were also carried out in the urban communities of Anthony and Las Cruces, New Mexico (Fig 1). El Paso is an urban city located in the far southwestern part of Texas bordering Mexico. El Paso city has a population of 681,728, while El Paso County has a population of 839,238 [24,25], in close proximity to two urban communities, the cities of Las Cruces and Anthony, both in Doña Ana County, NM. Las Cruces is located 45 miles northwest of El Paso with a population of 103,432 [26]. Anthony is located 18 miles northwest of El Paso with a population of 9,239 [27]. The other collection site, IMRS, is located 26 miles southwest of Van Horn, TX (121 miles from El Paso). IMRS has no permanent residents, and is used as a field research station by UTEP and as a camping site. Previously, we reported the prevalence of *T. cruzi* in IMRS [28]. The triatomine species were identified morphologically [29] and by PCR [30]. Insects were collected by our laboratory members using black light vane trap (BioQuip, Rancho Dominguez, CA) (S1 Fig), by hand collection using tweezers [31,32] (S1 Table), and by members of the communities by unreported *ad hoc* methods (e.g., household items such as cups, pans, plastic bags, etc.). A total of 225 triatomines were captured; 212 were preserved in 95% ethanol, whereas 13 were stored directly at -20°C until analysis. All insects were rinsed in a 1% sodium hypochlorite solution and dissected using sterile instruments in a class-II biosafety cabinet [33]. Dissection was done by
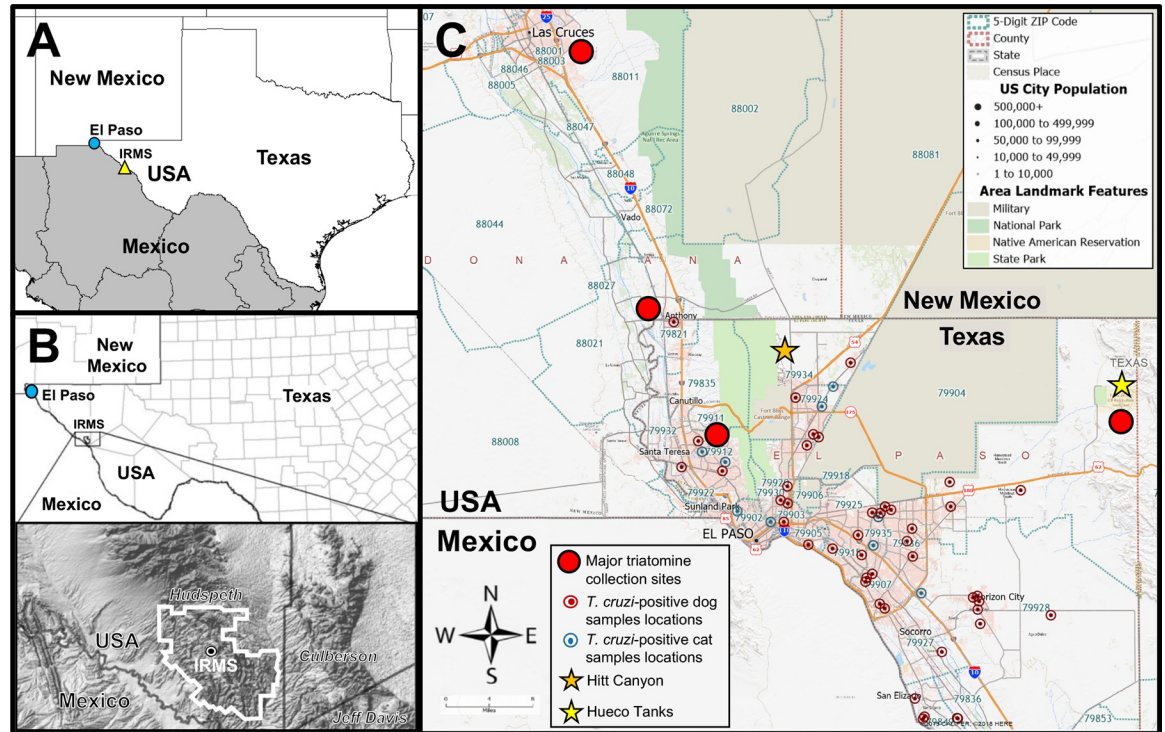
Fig 1. Sample collection sites in El Paso County and surrounding areas in Texas and New Mexico. (**A**) Major triatomine collection sites in or nearby El Paso County (blue circle) and at IRMS, TX (yellow triangle). (**B**) Detailed location and map of IMRS, TX. Inset: IRMS laboratory (encircled black dot) and the field station entire area (white line) are indicated. (**C**) Detailed map of distribution of collected samples in El Paso County, TX, and nearby areas in New Mexico (cities of Anthony and Las Cruces). Maps sourced from the U.S. Geological Survey (https://www.usgs.gov/) and generated with Maptitude 2019 (Caliper Software) and DIVA-GIS 7.5 (https://www.diva-gis.org/).

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carefully removing both pairs of wings, cutting off the connexivum to finally lift up the abdominal wall to reveal the hindgut. The triatomine hindgut was homogenized with a cordless pestle motor (Kimble) in 40 μL phosphate-buffered saline (PBS), pH 7.4. DNA extraction of all samples were done with High Pure PCR Template Preparation Kit (Roche) using the manufacturer's recommendation. The triatomine genetic material was used to identify the insect species by sequencing a PCR amplification product of mitochondrial DNA, as previously described [30]. Maps were generated with Maptitude 2019 (Caliper Software, Newton, MA) and DIVA-GIS 7.5 (https://www.diva-gis.org/).

Detection of *T. cruzi* in triatomine samples

The samples of triatomine hindguts were tested for *T. cruzi* genomic DNA by PCR amplification of a kinetoplastid DNA minicircle, as previously described with minor modifications [34]. Reactions consisted of 200 ng of DNA sample, primers 121 (5'-AAATAATGTACGGG(T/G) GAGATGCATGA-3') and 122 (5' GGTCGATTGGGGGTTGGTGTAATATA-3' at final concentration of 0.4 μM each, 12.5 μM 2X GoTaq Green Master Mix (Promega), and nuclease-free water to a final volume of 25 μL. Reactions were run in a 9902 Veriti PCR Thermal Cycler (Applied Biosystems), using the following conditions: incubation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec. Final extension at 72°C for 7 min and end with at 4°C hold. *T. cruzi* (Dm28c clone) genomic DNA positive control

was run in all PCR reactions. A positive result was determined by visualization of 330 bp band on a 2% agarose gel. The faint bands were reamplified; the PCR reactions were run again with a double concentration of genomic DNA and the PCR products that showed a very wide band (smear-like) were repeated diluting the amount of DNA to half. The homogeneity in *T. cruzi* detection (*i.e.*, equal proportion of positive outcomes in the PCR reactions) among the 4 locations was tested with a χ^2 test, setting $\alpha = 0.05$. The positive ratios in each location were compared using Cohen's D effect size [35]

Triatomine bloodmeal analysis

T. cruzi-positive triatomine samples were analyzed to determine the mammalian species on which they fed. Amplification of the 16S rDNA was accomplished by hemi-nested PCR, as previously described [36] with minor modifications. The first reaction contained 3 μL of DNA template from triatomine, a universal forward primer (VF) (ACC CNT CYM TGT NGC AAA AKR GTG) and a mammalian group-specific forward primer (MF) (CCT GTT TAC CAA AAA CAT CAC) at 0.25 μM final concentration, 12.5 μL 2X GoTaq Green Master mix, and nuclease-free water to a final volume of 25 μL. The secondary reaction contained 2 μL of the primary PCR product, mammalian group-specific reverse primer (MR) (AYT GTC GAT AKG RAC TCT WRA RTA) and MF at 0.5 μM final concentration, 12.5 μL 2X GoTaq Green Master mix, and nuclease-free water to a final volume of 25 μL. A positive triatomine control fed previously with rabbit blood was used in all reactions. Subsequent bi-directional sequencing was run on an ABI 3130x/Genetic analyzer using Bid Dye Terminator chemistries (Applied Biosystems), following the manufacture's recommendation. Complementary sequences were assembled and verified using CodonCode Aligner V 9.0.1 (CodonCode Corporation, Centerville, MA). Consensus sequences were compared with DNA nucleotide sequences in NCBI BLAST, using the blastn query. Query results with maximum identity equal to or higher than 95% and Expect (E) value equal to or lower than 0 were considered matches. Positive species hits were confirmed to be present in El Paso and surrounding areas based on species distribution using biodiversity databases [37,38]. A total of 41 samples presented mixed template sequencing traces that overlapped, making it impossible to use them. This is because it is known that triatomines may contain DNA from more than one mammalian bloodmeal. To resolve this, restriction enzymes were used for restriction fragment length polymorphism (RFLP) analysis, as described previously [36]. PCR products were singly or doubly digested with Anza 68 *BsuRI* and/or Anza 44 *AluI* (Invitrogen) for 15 min, at 37˚C, in a reaction containing 10 U of each enzyme, 1X Anza buffer, 2 μL PCR product and nuclease-free water to a final volume of 20 μL. Enzymes were heat-inactivated at 80˚C for 20 min prior to electrophoresis. Products were electrophoresed in a 1.5% agarose gel and visualized with iBright FL1500 imaging system (Thermo Fisher Scientific). Band sizes were estimated using iBright imaging software (Thermo Fisher Scientific) and values compared to controls of the most common mammals found during DNA sequencing (human, rat, mouse, dog, bat, cat, rabbit, horse, and squirrel) and to values previously published [36]. The Shannon-Wiener Index was calculated to compare the bloodmeal diversity at each site, as higher values suggested higher diversity in the site [39, 40], considering only those species confirmed as described above.

Feral and wild mammal sample collection and DNA extraction

The blood samples from feral or stray dogs and cats were collected from a local animal shelter. Each sample had capture location coordinates determined by GPS (S1 Table). Wild rodents were collected at the Hitt Canyon in the Franklin Mountains State Park in El Paso, TX, using baited Sherman and tomahawk traps. Trapped rodents were identified to species [29], euthanized at the site and the hearts were harvested for analysis. Heart tissues (20–30 mg) were

homogenized using gentleMACS M tubes and gentleMACS dissociator (Miltenyi Biotec) in 200 μL PBS. DNA was extracted and each sample was subject to *T. cruzi* detection, as described above.

T. cruzi DTU determination

All *T. cruzi* positive samples were subjected to additional reactions to determine the infecting parasite DTU by multilocus conventional PCR, as previously described [41]. This method was based on the amplification of different *T. cruzi* targets. The PCR targets were the following: the intergenic region of Spliced Leader (SL-IRac) to distinguish between TcI (150 bp), TcII, TcV and TcVI (157bp), and TcIII and TcIV (200bp) using primers UTCC (CGTACCAATATAGT ACAGAAACTG) and TCac (CTCCCCAGTGTGGCCTGGG). The SL-IR I-II target to differentiate TcI (350 bp) from TcII, TcV and TcVI (300 bp) using primers TCC (CCCCCCTCCCA GGCCACACTG), TC1 (GTGTCCGCCACCTCCTTCGGGCC) and TC2 (CCTGCAGGCAC ACGTGTGTGTG). The D7 domain of the 24Sα ribosomal DNA target to distinguish between TcII, TcIV and TcVI (140 bp), and TcIII and TcV (125 bp) in a heminested PCR using primers D75 (GCAGATCTTGGTTGGCGTAG) and D76 (GGTTCTCTGTTGCCCCTTTT) (first round) and D76 and D71 (AAGGTGCGTCGACAGTGTGG) (second round). Moreover, the A10 nuclear fragment target to differentiate TcII (580 bp) from TcVI (525 bp) in a heminested PCR using primers Pr1 (CCGCTAAGCAGTTCTGTCCATA) and P6 (GTGATCGCAGGAA ACGTGA) (first round) and Pr1 and Pr3M (CGTGGCATGGGGGTAATAAAGCA) (second round). The control PCR reactions were performed with 100 pg gDNA extracted from *T. cruzi*: Dm28c (TcI), Y (TcII), INPA 3663 (TcIII), CanIII (TcIV), LL014 (TcV), and CL Brener (TcVI). If a sample showed more than one expected band, the presence of more than one infecting parasite DTU was considered and the sample was genotyped as a mix infection.

Results

Triatomines collected and *T. cruzi* prevalence

A total of 225 triatomines were collected from four different sites, including 21 in El Paso, TX, 33 at IMRS, TX, 139 in Anthony, NM, and 32 in Las Cruces, NM (Fig 1; Table 1). The most common (98.2%, 221/225) species collected was *Triatoma rubida* across all sites, followed by *Triatoma gerstaeckeri* and *Triatoma protracta* (both 0.9%, 2/225), which were both collected in El Paso (Table 1). A total of 150 (66.7%) triatomine samples were positive for *T. cruzi* by PCR (Table 1), and of those 147 were *T. rubida*, two *T. gerstaeckeri*, and one *T. protracta*. The prevalence rate of *T. cruzi* in triatomines varied along collection locations ($\chi^2 = 12.8653$, p = 0.002) as follow: Las Cruces, NM (27/32, 84.4%), IMRS (24/33, 72.7%), Anthony, NM (91/139, 65.5%) and El Paso, TX, (5/17, 29.4%) (Table 1). Cohen's D effect size identified El Paso as having a lower prevalence rate than all other locations (Cohen's D large effect size >0.5) and Anthony as having a lower prevalence rate than Las Cruces (Cohen's D medium effect size >0.3) [35].

Triatomine bloodmeal analysis

The bloodmeal source was identified in 65.3% (98/150) of the *T. cruzi*-positive triatomines collected at three different sites, using two methodologies (DNA sequencing, n = 57, and RFLP, n = 41) (Table 2; S2 Table). Thirteen different mammalian host species were identified. The majority (n = 95; 96.9%) of bloodmeal sources were identified from *T. rubida*, one from *T. protracta*, and two from *T. gerstaeckeri* (Table 2). Furthermore, by bloodmeal DNA sequencing, we found that one of the *T. protracta* specimens collected in El Paso, TX, contained a single

Table 1. *T. cruzi*-positive triatomines and DTU genotyping of triatomines collected in the El Paso County, TX, and surrounding rural and urban communities in Texas and New Mexico.

Location	Species	*T. cruzi* status P/T [a] (%)	DTU			
			TcI	TcIV	TcI+TcIV	ND [b]
El Paso, TX						
	T. rubida	5/17 (29.4)	4	0	0	1
	T. protracta [c]	1/2 (50.0)	1	0	0	0
	T. gerstaeckeri [c]	2/2 (100.0)	2	0	0	0
IMRS, TX						
	T. rubida	24/33 (72.7)	22	1	0	1
Anthony, NM						
	T. rubida	91/139 (65.5)	84	1	5	1
Las Cruces, NM						
	T. rubida	27/32 (84.4)	25	0	2	0
	Total	**150/225 (66.7)**	**138**	**2**	**7**	**3**

a P/T, Positive for *T. cruzi* per total number of triatomines collected

b ND, Not determined

c Found in Hueco Tanks, near El Paso, TX

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bloodmeal from *Mus musculus*, whereas by RFLP, we observed that two *T. gerstaeckeri* specimens shared a mixed bloodmeal of dog (*Canis* sp.) and mouse (*Mus* sp.). However, unfortunately, we were unable to determine the species of the latter. The greatest blood feeding diversity was

Table 2. Identification of mammalian host species that served as a bloodmeal source for *T. cruzi*-infected triatomines in El Paso and IMRS, TX, and Anthony, NM, identified by DNA sequencing and RFLP analysis.

Location	DNA sequencing		RFLP [a]	
	Host species	Bloodmeal n	Host species	Bloodmeal n
El Paso, TX	*Mus musculus* (house mouse)	3 [b]	*Felis* sp. and *Mus* sp.	1
	Canis lupus familiaris (domestic dog)	2	*Canis* sp. and *Mus* sp.	2 [c]
IMRS, TX	*Neotoma leucodon* (white-throated woodrat)	4	*Neotoma* sp. and *Sylvilagus* sp. or *Lepus* sp.	6
	Homo sapiens (human)	2	*H. sapiens* and *Antrozous* sp.	1
	Antrozous pallidus (pallid bat)	2	*Mus* sp. and *Antrozous* sp.	4
			Mus sp. and *Sylvilagus* sp. or *Lepus* sp.	4
Anthony, NM	*Mus musculus* (house mouse)	19	*Canis* sp. and *Mus* sp.	4
	Homo sapiens (human)	5	*H. sapiens* and *Mus* sp.	2
	Canis lupus familiaris (domestic dog)	5	*Mus* sp. and *Sylvilagus* sp. or *Lepus* sp.	5
	Ammospermophilus interpres (Texas antelope squirrel)	3	*Mus* sp. and *Neotoma* sp.	9
	Sylvilagus audubonii (desert cottontail rabbit)	3	*Mus* sp. and *Antrozous* sp.	1
	Myotis lucifugus (little brown bat)	3	*Mus* sp. and *Ammospermophilus* sp.	1
	Lepus californicus (black-tailed jackrabbit)	2	*Ammospermophilus* sp. and *Sylvilagus* sp. or *Lepus* sp.	1
	Peromyscus maniculatus (deer mouse)	1		
	Perognathus merriami (Merriam's pocket mouse)	1		
	Felis catus (domestic cat)	1		
	Equus ferus caballus (domestic horse)	1		
Total		57		41

a This analysis was done based on the fragment size of the double-digestion with *BsuRI* and *AluI* of the 16S rDNA fragment obtained by PCR, and compared with already known sequences obtained here, and published data [36]. Based on this analysis, most of the digestion fragments had very close molecular sizes, which impaired the identification of the animal species.

b, c Out of the 98 bloodmeals listed in the table, 95 were identified from *T. rubida*, one from *T. protracta* ([b]), and two from *T. gerstaeckeri* ([c]).

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Table 3. Prevalence of *T. cruzi* infections and DTU genotyping in feral or stray dogs and cats collected in El Paso County, TX.

Species	*T. cruzi* status P/T [a]	DTU			
	(%)	TcI	TcIV	TcI+TcIV	ND [b]
Canis lupus (domestic dog)	43/95 (45.3)	30	9	1	3
Felis catus (domestic cat)	7/24 (29.2)	2	1	0	4
Total	50/119 (42.0)	32	10	1	7

a P/T, Positive for *T. cruzi* per total number of triatomines collected

b ND, Not determined

detected in Anthony, NM, where 11 different host species served as a source of blood and the value for the Shannon-Weiner Index was 1.67, higher than the values 1.34 and 0.90 found in IMRS and El Paso, respectively. The most frequently host species fed upon by triatomines in El Paso and Anthony was the house mouse, while woodrats were the most frequently preferred host in IMRS. Interestingly, humans were identified as a source of blood for triatomines in Anthony and IMRS (n = 7 and n = 3, respectively). Canid blood was also commonly found in triatomines collected in El Paso and Anthony (n = 4 and n = 10, respectively) (Table 2). The source of blood for 25 triatomines could not be identified by either of the two methods used in this study.

T. cruzi infection in feral cats and dogs, and wild animals

A total of 95 and 24 blood samples were collected from stray domestic canines and felines, respectively, within the El Paso urban area (Table 3). Among these samples, 45.3% (43/95) of the dogs and 29.2% of the cats (7/24) were positive for *T. cruzi* by PCR (Table 3). Additionally, at the Hitt Canyon of the Franklin Mountains State Park, located in El Paso, TX, a total of seven wild animals' heart samples were collected from two *Chaetodipus intermedius*, and one each of *Peromyscus eremicus*, *Xerospermophilus spilosoma*, *Reithrodontomys megalotis*, *Urocyon cinereoargenteus*, and *Canis latrans*. When analyzed for *T. cruzi* infection by PCR, all but two from *C. intermedius* (rock pocket mouse) were positive for *T. cruzi* (Table 4).

T. cruzi DTUs genotyping

Among all of the *T. cruzi*-infected triatomines, and feral and wild animals, the parasite was successfully genotyped in 95.1% of the samples (195/205; Tables 1, 3 and 4). Of the total 150 *T. cruzi*-positive triatomines, genotyping revealed that 138 were DTU TcI across all sites, seven from El Paso, 22 from IMRS, 84 from Anthony, and 25 from Las Cruces (Table 1). In addition, two triatomines, one from IMRS and one from Anthony, showed TcIV infection. The

Table 4. Prevalence of *T. cruzi* infection and DTU genotyping in wild animals collected at Hitt Canyon, El Paso County, TX.

Species (Popular name)	n	*T. cruzi*-positive	DTU
Chaetodipus intermedius (rock pocket mouse)	2	0	NA
Peromyscus eremicus (cactus mouse)	1	1	TcI+TcIV
Xerospermophilus spilosoma (spotted ground squirrel)	1	1	TcI+TcIV
Reithrodontomys megalotis (western harvest mouse)	1	1	TcI
Urocyon cinereoargenteus (gray fox)	1	1	TcI
Canis latrans (coyote)	1	1	TcI
Total	**7**	**5**	

a NA, Not applicable

genotyping also revealed that seven triatomines had mixed TcI and TcIV (TcI+TcIV) infection (five from Anthony and two from Las Cruces) (Table 1). In the case of feral dogs in El Paso area, TcI was found in 30 animals. TcIV was found in nine canines and only one canine was found to have a mixed TcI+TcIV infection. In felines, TcI was found in two animals and TcIV in one animal (Table 3). Finally, in the wild animals, TcI infection was found in three animals (*R. megalotis*, *U. cinereoargenteus* and *C. latrans*) and two TcI+TcIV mixed infection were found in two animals (*P. eremicus and X. spilosoma)* (Table 4).

Discussion

The prevalence of *T. cruzi* infection in humans and animals in the U.S. has been recently more studied, since it has become an emerging public health threat in the southeast and southwest regions [9,16,19,22,23,42–47]. In this study, triatomines and blood samples from feral or stray dogs and cats, and wild animals were collected at four different sites in El Paso County and surrounding areas in TX and NM. These samples were tested for *T. cruzi* infection, and positive samples were genotyped to determine parasite DTUs. Moreover, triatomine bloodmeal analysis was performed on *T. cruzi*-positive samples to identify mammalian host species. This is the first study that determined the prevalence of *T. cruzi* infection in feral dogs and cats, wild animals, and triatomine vectors in El Paso County, and surrounding areas in Texas and New Mexico. It is important to highlight that this study also identified the species of regional triatomines, determined the *T. cruzi* infection rates in feral and wild animals, genotyped infecting *T. cruzi* parasites, and characterized the triatomine bloodmeals, therefore identifying other potential reservoirs of the parasite.

Three different species of triatomines were collected during this study. *T. rubida* was the most common species, followed by two species with similar low frequency rate of capture, *T. gerstaeckeri* and *T. protracta.* These two species with low frequency collection rates were captured at sites in an area named Hueco Tanks, a low rocky mountain rural community located in far east El Paso and worldwide well-known climbing and bouldering site, visited by hundreds of people every year (Fig 1). *T. gerstaeckeri* is the most frequently collected species in the U.S., mostly found in southeastern Texas, with a cumulative *T. cruzi* infection rate of 57.7%, while *T. protracta* has been reported in California, Arizona, New Mexico, and the southern border of Texas with Mexico, with an overall *T. cruzi* infection rate of 17.5% [2]. In the case of *T. rubida*, this species has been found from western Texas to southern California with a very low cumulative infection rate of 7.2% (much lower rate than what was found in this study) [2]. The present study is the first to report the presence of *T. gerstaeckeri* and *T. protracta* in El Paso County and surrounding areas. In the case of *T. rubida*, a few specimens have been reported since 1949 in El Paso as well as in Ciudad Juarez, a city across the border in Mexico [2,44,48,49]. The only other *Triatoma* sp. reported in El Paso is *Triatoma indictiva*, but no infection status or the exact collection location was available [2]. As for Las Cruces and Anthony, NM, *T. protracta* and *T. rubida* have been reported, but these reports belong to the Doña Ana County, where these two cities are located, and no exact location has been reported [2]. Here, *T. rubida* was also the only species found at IMRS, which is the same species reported by our group in 2015, when 39 *T. rubida* specimens were identified [28]. Studies on the biology behavior and spatial distribution of *T. rubida*, the most frequently collected species in this study, indicated that this species inhabits environments in close association with their hosts, which includes wild and domestic animals, as well as humans and they are extremely attracted to host-emitted cues, such as body odors, CO_2, moisture and heat as well as light sources [2,44,50–53]. All of these factors can be found in human residences. Remarkably, all residence locations where triatomines were collected were within the limits of the city of El

Paso, next to mountains in semi-rural areas. Furthermore, in El Paso, all residences had domestic dogs and the residents found the triatomines in close proximity to where their dogs slept. It has been reported that dogs are a risk factor for triatomine occurrence in residences forming a transmission bridge for *T. cruzi* between human and infected dogs [9,15,16,54]. Thus, a possible explanation for the prevalence of triatomine among residences within city limits of El Paso is that triatomines are attracted to dogs, humans, and light sources. This is also corroborated for triatomines collected from Anthony and Las Cruces, NM, which were collected around lights located in porches and outside buildings. Additionally, studies about the feeding and defecation behavior of *T. rubida* indicated this species as an efficient vector [50]. This is because *T. rubida* defecates while taking a bloodmeal and it has been proposed that triatomine species that defecate sooner than ten minutes after taking a bloodmeal are effective *T. cruzi* transmitter, such as the case for *T. rubida* [50, 55–57]. These findings suggest *T. rubida* poses a transmission risk of *T. cruzi* in El Paso County and surrounding areas, based on distribution, habitat, and feeding behavior.

Of the 225 triatomines collected and analyzed, 150 (66.7%) were positive for *T. cruzi*. This infection rate was within the range reported during similar studies conducted in the southern region of the U.S. (range from 41.5 to 80.6% in studies in Texas, Louisiana, and Arizona) [9,11,16,28,43,58–62]. While it is not possible to quantitatively compare infections rates among locations that were sampled using different methodologies, the potential biases resulting from such differences can be inferred to be small enough to allow qualitative comparisons [63]. In this study, Las Cruces, NM, had the highest infection rate (84.4%), which was higher than rates published previously in the southwest, followed by Anthony, NM (65.5%). Interestingly, there has not been any published studies of this nature in New Mexico, even though triatomines have been widely reported in that state [2]. El Paso County had the lowest infection rate of triatomines (8/21, 38.1%) (Table 1). While there is no clear explanation for the lower rate found in El Paso, it is possible that the small number of samples obtained in this location is preventing an accurate estimation of the infection rate. It is possible that the collection of more samples in the future will allow for a more robust estimation of the prevalence rate. Finally, the infection rate at IMRS (72.7%) was higher (15.4% increase) than that reported by our group in the same area in 2015 [28]. These findings demonstrate an active presence of *T. cruzi* in triatomines collected in and around El Paso County, TX.

Triatomines are obligate hematophagous and they can feed on any animal, but only mammals are susceptible to *T. cruzi* infection [64]. In our study, we used a methodology that allowed for the identification of mammalian host species as the source of triatomine bloodmeals. We only focused on the *T. cruzi*-infected insects for the purpose of linking infected triatomines with possible hosts that could have been infected by the insect. Therefore, the mammalian species source of bloodmeals were identified for 98 (79.7%) out of 123 *T. cruzi*-positive triatomines collected in residences or in the wild, and immediately immersed in 95% ethanol or frozen until processing. Twenty-seven triatomines from Las Cruces were not taken into consideration for the bloodmeal analysis (Table 2) because they were kept alive in the lab by feeding them rabbit blood. Our first approach detected single bloodmeals in 57 triatomine samples by DNA sequencing. Forty-one triatomine samples resulted in unreadable sequences that contain overlapping base reads (Table 2). This is because it is known that triatomines may contain DNA from more than one mammalian species [11,36,65]. Thus, we opted for a second approach to analyze the DNA by RFLP analysis [36]. By using this method, the mammalian blood source from the 41 aforementioned triatomines, was identified. Finally, failure in the identification of the mammalian bloodmeal source in 25 triatomine samples by both PCR and RFLP analysis could be attributed to: (i) triatomines feeding on non-mammalian hosts such as birds, thus non-amplifiable in the 16S rDNA mammal-specific PCR reaction; or (ii) the lack of

a recent bloodmeal, allowing for a degree of blood degradation products inside the insects' midgut that made the identification by RFLP unfeasible, as previously reported [66–69]. The most diverse source of host bloodmeal were found in Anthony, where triatomine fed on 11 different species of mammals and a higher Shannon-Wiener Index in contrast to El Paso and IMRS, where triatomines fed on 3 and 5 different species, respectively. This may be explained by the fact that Anthony is a semi-rural city with the greatest mammal diversity compared to the other sites. Among the most important results, humans were identified as the source of blood for 7 and 3 triatomines in Anthony and IMRS, respectively, raising the concern for the risk of Chagas disease transmission in these localities, especially at IMRS which is used as a camping and biology field station site. Of all locations, only in El Paso have there been reported cases of *T. cruzi*-positive in blood donors, but no cases of autochthonous transmission have been reported [70]. Moreover, domestic dog, domestic cat, and house mouse were found to be a source of blood for triatomines. These animals are important because they live near humans and extensive studies have shown that these animals pose a risk as a source for possible human infection [71,72]. These findings present evidence for potential vector-borne transmission of Chagas disease for humans as well as domestic animals in El Paso County and surrounding areas.

Stray or feral domestic dogs and cats, and wild animals were also tested for *T. cruzi*. Domestic dogs are considered as an important reservoir for *T. cruzi* in Latin America [17,71,73–81]. It has been proposed that dogs are more likely to acquire *T. cruzi* infection due to ingestion of infected triatomines, thus allowing for oral transmission, and that dogs usually sleep outside, increasing therefore the chances of triatomine contact [9,10,17,47,54,82]. Moreover, infection in dogs has been reported from several southern states, including Texas and New Mexico [42,47]. Another animal that lives in close contact with humans are cats. They have been identified as potential sentinels for many infectious diseases because of the environments they share with humans [45]. However, the role of cats in relation to the maintenance and transmissibility of *T. cruzi* has not been extensively studied and is not fully understood; the very few studies conducted found a very low prevalence of infection [46,83–86]. In this study, 45.3% (43/95) of feral dogs sampled were positive for *T. cruzi*, while 29.2% (7/24) of the feral cats were positive for the parasite (Table 3). Samples from dogs and cats were collected from all over the inner city of El Paso. Furthermore, 71.4% (5/7) of the wild animals tested were positive for *T. cruzi*, thus suggesting an important reservoir role. All of the positive species have been previously reported in Texas as *T. cruzi* reservoirs [87–90]. Interestingly, triatomine traps were set at sites where *T. cruzi*-positive feral dogs and cats, and wild animals were captured, but attempts to collect triatomine at these sites were not successful. This might indicate that the infection in these animals was acquired congenitally. One of the limitations of this part of the study was that data for the age of animals was not available. Such data would have allowed us to analyze the odds of an animal being infected congenitally or through vector transmission, since older animals have a longer opportunity to be exposed to the vector [16,91]. These findings and the substantial number of reported cases (dogs and cats) in Texas and New Mexico discussed in this study may indicate that Chagas disease may occur among feral dogs and cats in the region and that a transmission cycle between vector and animals may have already been established in the western part of Texas.

Finally, the DTUs of all *T. cruzi*-positive samples were genotyped with a successful rate of 95.1%. DTUs are important markers for clinical, ecological, and epidemiological features and range from TcI to TcVI and TcBat [92]. Here, TcI was the most predominant genotype, followed by TcIV and some *T. cruzi* had a mixed TcI+TcIV infection. Among feral dogs and cats, the equal distribution of DTUs confirmed that there was no difference among them as hosts. TcI is found throughout the Americas with domestic and sylvatic transmission cycles and its

associated with chronic Chagas cardiomyopathy [93, 94]. The finding that the most predominant DTU was TcI is an alarming public health concern since chronic Chagas disease is the most challenging form of the disease to be diagnosed and the harder to treat, with possible irreversible hearth damage [95–97]. On the other hand, TcIV is more involved in sylvatic transmission cycles in northern part of South America and the USA, but its clinical association is not well understood and its risk to humans is unknown [98]. These findings are consistent with the documented DTU characterization of *T. cruzi* across Texas and USA, where both DTUs have been reported in humans and an extensive range of mammalian species [2,99].

The rates and the observations that *T. cruzi* infection rates ranged from moderate to high level in domestic and wild animals support an active enzootic/endemic transmission cycle in the El Paso and surrounding communities. The observation that the most collected triatomine specimens were *T. rubida*, which is considered an efficient vector, and the most observed DTU was TcI, which is causative of the very harmful chronic Chagas cardiomyopathy, indicates a high health risk for these communities that needs to be taken into consideration by the respective health authorities. Additionally, the finding of human, canine and feline bloodmeals in these triatomines suggests the possibility of autochthonous parasite transmission in humans as well as in peridomestic or feral animals in the studied areas. More extensive studies are needed, particularly in the high-populated El Paso County area to better understand the involvement of humans and feral and wild animals in the transmission cycle as well as in the burden of Chagas disease.

Supporting information

S1 Fig. Black light vane trap used in this study to collect triatomines.
(PDF)

S1 Table. Coordinates of animal capture locations.
(XLSX)

S2 Table. Triatomine and bloodmeal species identified.
(XLSX)

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