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Research paper

Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for *Trypanosoma cruzi* infection and discrete typing units

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ABSTRACT

Across the Americas, triatomine insects harbor diverse strains of *Trypanosoma cruzi* (*T. cruzi*), agent of Chagas disease. Geographic patterns of vector infection and parasite strain associations, especially in vectors encountered by the public, may be useful in assessing entomological risk, but are largely unknown across the US. We collected *Triatoma* spp. from across the US (mainly Texas), in part using a citizen science initiative, and amplified *T. cruzi* DNA to determine infection prevalence and parasite discrete typing units (DTUs). We found 54.4% infection prevalence in 1510 triatomines of 6 species; prevalence in adult *T. gerstaeckeri* (63.3%; $n = 897$) and *T. lecticularia* (66.7%; $n = 66$) was greater than in *T. sanguisuga* (47.6%; $n = 315$), *T. indictiva* (47.8% $n = 67$), *T. rubida* (14.1%; $n = 64$), and *T. protracta* (10.5%; $n = 19$). The odds of infection in adults were 9.73 times higher than in nymphs (95% CI 4.46–25.83). PCR of the spliced leader intergenic region (SL-IR) and/or the putative lathosterol/episterol oxidase TcSC5D gene revealed exclusively *T. cruzi* DTUs TcI and TcIV; 5.5% of *T. cruzi*-positive samples were not successfully typed. *T. gerstaeckeri* ($n = 548$) were more frequently infected with TcI (53.9%) than TcIV (34.4%), and 11.9% showed mixed TcI/TcIV infections. In contrast, *T. sanguisuga* ($n = 135$) were more frequently infected with TcIV (79.3%) than TcI (15.6%), and 5.2% showed mixed infections. Relative abundance of parasite DTUs varied spatially, with both TcI and TcIV co-circulating in vectors in central Texas, while TcIV predominated in northern Texas. Given prior findings implicating TcI in human disease and TcI and TcIV in animal disease in the US, knowledge of spatial distribution of *T. cruzi* infection and DTUs in vectors is important to understanding public and veterinary health risk of *T. cruzi* infection.

1. Introduction

Throughout the Americas, the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) is responsible for an estimated burden of Chagas disease exceeding 5.7 million people (World Health Organization, 2015). Transmission is primarily through the infective feces of triatomine insects, although transmission can also occur congenitally, through organ transplant and blood transfusion, and through consumption of contaminated food and drink (Bern et al., 2011). Infection with *T. cruzi* ranges from asymptomatic to clinical presentation of acute or chronic cardiomyopathy and, less commonly, gastrointestinal complications (Bern et al., 2011).

T. cruzi exhibits remarkable genetic variation (Miles et al., 2009; Zingales et al., 2009, 2012), and current classification delineates six major discrete typing units (DTUs), TcI - TcVI, and a seventh, TcBat lineage, each with particular ecological and epidemiological associations (Zingales et al., 2012). In the Southern Cone region of South America, for example, TcII, TcV, and TcVI have been mainly documented in domestic transmission cycles; in contrast, TcIII and TcIV are more typically associated with sylvatic transmission cycles in Brazil and northern South America (Miles et al., 2009; Zingales et al., 2012). TcI is the most genetically diverse DTU and is found throughout the Americas with variable domestic and sylvatic associations (Zingales et al., 2012), which has resulted in additional proposed subdivisions (Zumaya-Estrada et al., 2012). Understanding the effect of parasite strain on disease progression is complicated by co-infections, transmission mode, and individual host immune function (Messenger et al., 2015a; Zingales et al., 2012).

In the southern US, *T. cruzi* actively circulates through vector, wildlife, and domestic dog populations (Brown et al., 2010; Burkholder

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et al., 1980; Curtis-Robles et al., 2015, 2016; Kjos et al., 2008, 2009), and autochthonous human Chagas disease is also documented in the US (Cantey et al., 2012; Dorn et al., 2007; Garcia et al., 2015). Standard diagnostic approaches are typically based on antibody detection and do not determine parasite strain, so the increasing diagnoses of human and animal *T. cruzi* infections in the US are not generally accompanied by a concomitant increase in the knowledge of which strains are implicated in disease. Strain-typing approaches that have been applied to samples from the US include methods based on enzyme profiles (Barnabé et al., 2001; Beard et al., 1988), PCR product sizes of several genetic targets (Garcia et al., 2017; Herrera et al., 2015; Roellig et al., 2008), and DNA sequence analysis (Buhaya et al., 2015; Curtis-Robles et al., 2016; Garcia et al., 2017; Herrera et al., 2015; Shender et al., 2016). There have been seven definitively typed human infections in the US, all of which were TcI (Garcia et al., 2017; Roellig et al., 2008). Limited studies of dog and wildlife populations have revealed an association of TcI with opossums and TcIV with dogs and raccoons (Curtis-Robles et al., 2016; Roellig et al., 2008). Recently, TcII was found in small rodents from Louisiana (Herrera et al., 2015), and insects in California were found to harbor isolates closely related to the TcII and TcVI group members (Hwang et al., 2010). Triatomine vectors have been found infected with TcI in a limited number of samples from California, Florida, Georgia, Louisiana, and Texas (Barnabé et al., 2001; Beard et al., 1988; Buhaya et al., 2015; Herrera et al., 2015; Roellig et al., 2008; Shender et al., 2016); TcIV in triatomines has been documented rarely in California, Georgia, and Texas (Barnabé et al., 2001; Roellig et al., 2008; Shender et al., 2016).

Despite the recognized ecological and potential epidemiological importance of *T. cruzi* genetic variation, the majority of data are from South America (Brenière et al., 2016). Few studies have determined the DTUs of parasite isolates from various hosts in the US, and none have examined relative spatial distribution across a broad area. Knowledge of the prevalence and distribution of *T. cruzi* in US triatomine vectors—particularly those encountered by humans in peridomestic settings—would allow a greater understanding of disease risk and potential consequences. In this study, we determined *T. cruzi* infection prevalence in six triatomine species from Texas and other states. We analyzed samples using multiple PCR assays and assessed risk factors for infection through logistic regression. Given interests in vector associations and spatial distributions of *T. cruzi* DTUs in the US, we compared DTUs found in the two most frequently collected species and mapped occurrences of *T. cruzi* DTUs. We discuss the results with particular attention to potential drivers of differential DTU occurrence in species of triatomines found across the US.

2. Materials and methods

2.1. Sample collection and preparation

Triatomine specimens were collected from April 2013–November 2016 via citizens through a citizen science program (Curtis-Robles et al., 2015) and via our laboratory members using traditional entomological techniques (black light, mercury vapor light, and active searching of environments). Triatomines were morphologically identified to species (Lent and Wygodzinsky, 1979), sexed, and dissected using sterile instruments after specimens were soaked with 10% bleach solution and rinsed with distilled water to reduce risk of contamination with exogenous DNA. Dissections were conducted by carefully cutting off the connexivum, snipping 1–2 terminal abdominal segments, and removing the dorsal portion of the abdominal wall to reveal and remove the hindguts. In cases where hindguts were desiccated, the snipped terminal segments were also included in the sample to increase the sample amount. DNA from triatomine hindguts was extracted using commercially-available extraction kits (Omega E.Z.N.A. Tissue DNA Kit (a spin column-based kit), Omega Bio-tek, Norcross, GA for almost all (93.0%; Table S1) samples collected in 2013–2014; KingFisher Cell and Tissue kit (a magnetic-particle-based kit), Thermo Fisher Scientific, Waltham, MA for almost all (96.1%; Table S1) samples collected in 2015–2016).

2.2. Detection of T. cruzi infection

T. cruzi infection status was determined by amplification of a 166-bp region of repetitive nuclear satellite DNA using a TaqMan qPCR reaction with Cruzi 1/Cruzi 2 primers and Cruzi 3 probe (Duffy et al., 2013; Piron et al., 2007). This approach has previously been shown as both sensitive and specific for *T. cruzi* (Schijman et al., 2011). Reactions consisted of 5 μL of template DNA, primers at a final concentration of 0.75 μM each, 0.25 μM of probe, and iTaq Universal Probes Supermix (Bio-Rad Laboratories, Hercules, CA), in a total volume of 20 μL run on a Stratagene MxPro3000 instrument (Agilent Technologies, Santa Clara, CA), following previously described thermocycling parameters (Duffy et al., 2013), except with a reduced, 3-min, initial denaturation. Our internal laboratory validations—experiments using serial dilutions of *T. cruzi* TcIV DNA from a *T. gerstaeckeri* specimen collected in Texas to calculate limit of detection and PCR efficiency for this assay—defined samples with cycle threshold (Ct) values of < 33 as positive, and samples with Ct values of > 35 as negative. Samples with Ct values of 33–35 were considered equivocal and final infection status was based on the result on the SL-IR qPCR assay (see below). No-template controls were included in each set of DNA extractions, and molecular grade water was included as negative controls in all PCRs. All PCRs were run with a *T. cruzi* positive control, including DNA extracted from Sylvio-X10 CL4 (ATCC 50800, American Type Culture Collection) or *T. cruzi*-positive field-collected samples from triatomines, dogs, and wildlife (infections with either TcI or TcIV).

2.3. T. cruzi DTU determination

All *T. cruzi* positive and equivocal samples were subjected to additional reactions to amplify the putative lathosterol/episterol oxidase gene (TcSC5D) and/or nuclear spliced leader intergenic region (SL-IR) for DTU typing of *T. cruzi*. Originally, we attempted amplification of the TcSC5D region and sequenced the product to determine the DTU of *T. cruzi* in the vector hindgut DNA extracts (Cosentino and Agüero, 2012). During the course of this research, a probe-based multiplex qPCR based on SL-IR amplification was published (Cura et al., 2015). Subsequent to establishing this DTU-typing qPCR in our lab, we ran all previously untyped *T. cruzi* positive and equivocal samples, as well as a subset of samples successfully typed using the TcSC5D PCR, using this method.

2.3.1. TcSC5D gene amplification for DTU determination

Initially, we attempted amplification of the TcSC5D gene (Cosentino and Agüero, 2012) of any sample generating a positive *T. cruzi* result on the Cruzi 1/2/3 qPCR. However, *T. cruzi*-positive samples with relatively high Ct value (indicative of low parasite burdens) were not successfully amplified with the TcSD5D assay, perhaps reflecting the fact that the TcSC5D PCR was developed using *T. cruzi* pure cultures (Cosentino and Agüero, 2012) and seems to be less successful in analyzing field-collected samples with mixed populations of host and parasite DNA (Cominetti et al., 2014; Curtis-Robles et al., 2016; although see Buhaya et al., 2015). Thereafter, we focused on TcSC5D gene amplification from samples with greater concentrations of *T. cruzi* DNA, using a cut-off of Ct values of < 17 on the TaqMan qPCR. Reactions consisted of 1 μL extracted DNA, 0.75 μM of each primer, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a total volume of 15 μL. The 832-bp amplicons were visualized on 1.5% agarose gels stained with ethidium bromide. In some cases, samples that did not amplify were tested again on this assay using the same conditions or a 1:10 dilution of the DNA template. Amplicons were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and bi-directionally sequenced using Sanger sequencing (Eton Bioscience, Inc., San