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Source: Journal of Wildlife Diseases, 54(4) : 814-818

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/2017-09-223>

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Identification of White-tailed Deer (*Odocoileus virginianus*) as a Novel Reservoir Species for *Trypanosoma cruzi* in Texas, USA

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ABSTRACT: Chagas disease, a vector-borne parasitic infection caused by *Trypanosoma cruzi*, represents a significant source of morbidity and mortality in the Americas. Mammalian reservoir species play a large role in propagating the sylvatic transmission cycle of this disease, and this cycle can spill over, resulting in human infections. Our understanding of the wildlife species implicated in propagating this transmission cycle is incomplete. We investigated white-tailed deer (*Odocoileus virginianus*) as a potential novel reservoir for this parasite. Only one of the 314 hunter-harvested deer hearts collected across Texas, was PCR-positive (0.3%) for *T. cruzi*. This finding has potential implications for deer hunters, because it indicates that there might be a risk of blood-borne transmission during the field-dressing process. Hunters should be strongly encouraged to wear gloves and other personal protective equipment when handling carcasses to prevent exposure to infected blood.

Key words: Chagas disease, reservoir species, *Trypanosoma cruzi*, white-tailed deer, zoonosis.

Chagas disease is a vector-borne parasitic infection caused by the protozoan *Trypanosoma cruzi*. Following infection, the acute stage of human Chagas disease is commonly characterized as asymptomatic (Bern et al. 2011). About 30% of infected individuals will develop progressive cardiac disease, often leading to heart failure and death, over the course of several decades. Cardiac disease is not limited to human infection, because other mammalian species have demonstrated heart failure as a result of *T. cruzi* infection (Andrade et al. 1997). However, our understanding of clinical outcomes in sylvatic reservoir species is considerably limited.

Several species of Triatominae serve as the primary vector for this parasite. Transmission occurs when an infected triatomine

insect takes a blood meal and subsequently defecates the parasite onto the host's skin. If the infective feces are inoculated into an abrasion or a mucus membrane, then systemic infection can occur. In addition to vector-borne transmission, other notable sources of infection have been identified, such as consumption of parasite-contaminated fruit and vegetable juices (Filigheddu et al. 2017) and consumption of infected insects (de Noya and Gonzalez 2015). The latter route might represent a major source of infection in wild and domestic animal reservoir species.

Mammalian reservoir species play a large role in propagating sylvatic transmission; over 180 mammal species have been implicated as a reservoir worldwide (World Health Organization 2002). In Texas, at least 24 species have been implicated in sylvatic transmission (Gunter et al. 2017a). These animals have the ability to transmit the parasite congenitally to their offspring, allowing for robust transmission to continue in the absence of competent vector species (Rodríguez-Morales et al. 2011). Robust sylvatic transmission cycles are of concern as spillover from the sylvatic transmission cycle has been proposed as the major source of human infection in the US (Garcia et al. 2014; Gunter et al. 2017b). Additionally, in Texas, domestic canines have been found to serve as a reservoir host for Chagas disease, and might serve to bridge the gap between domestic and sylvatic transmission (Tenney et al. 2014).

Our understanding of the sylvatic transmission cycle of this parasite among wild animals remains in its infancy. Previous research identified opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and woodrats (*Neotoma*

toma sp.) as the major species implicated in propagating sylvatic transmission cycles in North America. Notably lacking from the previous investigations was evaluation of white-tailed deer (WTD; *Odocoileus virginianus*) as a potential reservoir species. Furthermore, recent analysis of triatomine bugs collected in Texas identified WTD as a source of blood meals, indicating the potential for these animals to serve as a reservoir species (Gorchakov et al. 2016). With over 500,000 WTD hunted annually in Texas alone, this species is the most commonly hunted big game animal in the state (Purvis 2017).

We believe hunters to be a particularly vulnerable population due to increased contact with vectors and wildlife reservoirs, thereby increasing the risk of infection by interrupting the sylvatic transmission cycle. The presence of *T. cruzi* in this animal species could introduce the potential for blood-borne transmission during field-dressing of deer if hunters do not wear proper protective equipment. To determine the risk of exposure to hunters, we investigated WTD as a novel reservoir for the parasite *T. cruzi*.

Through a citizen-science campaign, in collaboration with private hunting organizations and Texas Parks and Wildlife, we collected hunter-harvested deer hearts from 14 Wildlife Management Areas and private hunting lands in seven of the nine ecological regions of Texas (Fig. 1). Hunters were given a collection kit including a specimen bag, gloves, and intake form prior to the start of the hunt. They were asked to collect the entire heart and fill out basic information on the animal, including location, gender, estimated age (juvenile or adult), date and time of collection, and the time it was put into preservative. Specimens were then taken to either Texas Parks and Wildlife employees or to a check-point run by Baylor College of Medicine staff where the tissue was preserved by either submerging it in 10% neutral buffered formalin or freezing it at -80°C .

Once in the laboratory, cardiac samples were bisected on the lateral plane to reveal all four chambers of the heart. An equal-sized sample of muscle tissue was taken from the

apex, left ventricle outer wall, and interventricular septum from each heart. Tissue from the three anatomical locations manually homogenized together into a 25 mg sample. In the event samples could not be taken from all three anatomical locations, an apex sample of 25 mg was used. Formalin-fixed tissue samples were then washed in phosphate-buffered saline and frozen at -80°C for at least 24 h prior to DNA extraction.

We extracted DNA using the DNeasy Blood and Tissue kit (QIAGEN, Valencia, California, USA). Briefly, specimens were placed in lysis buffer and incubated at 56°C in a rotisserie-style incubator for either 24 h or 96 h for frozen and fixed tissue, respectively. Formalin-fixed tissue samples were then incubated for 1 h at 90°C to reverse formalin cross-linking. The remainder of the extraction was performed per kit protocol. Specimens were stored at -80°C until DNA amplification by PCR could be performed.

Purified DNA specimens were tested for the presence of *T. cruzi* DNA by real-time PCR methods using a ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, California, USA). We amplified *T. cruzi* DNA by targeting repetitive satellite DNA sequence as previously described (Schijman et al. 2011). TaqMan Fast Advanced Master Mix (Applied Biosystems) was used according to manufacturer's protocol. Each sample was tested for amplification of actin, a housekeeping gene, as an internal control to ensure adequate integrity of the DNA (Piorkowski et al. 2014; Table 1). Positive and negative controls were included for both *T. cruzi* and actin with every reaction to ensure that amplification was occurring as expected and that contamination was not occurring.

During the 2015 Texas WTD hunting season, a mixed cohort of 314 hearts from WTD (97.7%) and mule deer (*Odocoileus hemionus*; 2.3%) were collected. This cohort was comprised of 44% male deer, and 81% of the samples were collected from adult animals. Tissue samples were either preserved by fixation in 10% neutral buffered formalin (57%), frozen at -80°C (30%), or both (13%). The average time between harvesting

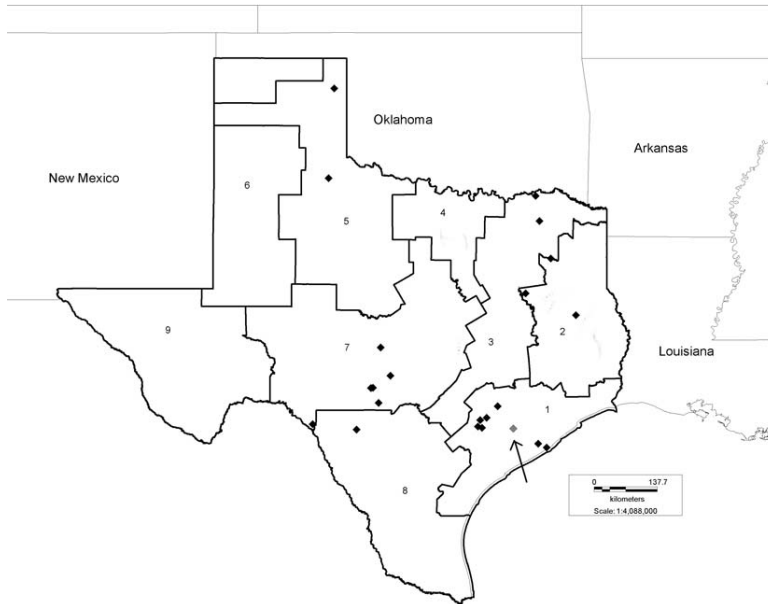


FIGURE 1. Locations from which hunter-harvested white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) hearts were collected for molecular detection of *Trypanosoma cruzi*. Samples were collected from six of the nine ecologic regions in Texas, USA with the following percent of the 314 samples collected from each region: 1, 44%; 2, 5%; 3, 3%; 5, 18%; 7, 28%; 8, 1%. The location of the Chagas-positive white-tailed deer collected in Moravia, Texas is indicated by an arrow.

of tissue and preservation by either freezing or formalin fixation was 3 h and 15 min (range: 0.5 h to 14.5 h). Our investigation revealed one out of the 314 (0.03%; 95% confidence interval=0.0006–0.2) samples had PCR evidence of infection with *T. cruzi* parasites. This positive heart was harvested from an adult female WTD in Moravia, Texas and was preserved by freezing.

For confirmation, endpoint PCR was run using TCZ primers for a repeat region of satellite *T. cruzi* DNA (Moser et al. 1989). Amplification was conducted in T100 Thermal Cycler (Bio-Rad, Hercules, California, USA) following the manufacturer's protocol for Phusion Hot Start II (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with positive and negative controls included. We

TABLE 1. The PCR amplification methods employed for the detection of *Trypanosoma cruzi* DNA in cardiac tissue from hunter-harvested white-tailed deer (*Odocoileus virginianus*) and controls, including targeted genome regions, the primer and probe sequences, the PCR set-up and number of cycles, and references.

Target	PCR	Target	Primer	Cycles	Reference
<i>T. cruzi</i>	Real-time	Satellite-DNA	Cruzi 1: 5' ASTCGGCTGATCGTTTTTCGA 3' Cruzi 2: 5' AATTCTCTCCAAGCAGCGGATA 3' TaqMan probe Cruzi 3: 5' CACACACTGGACACCAA 3'	45	Schijman et al. 2011
Actin	Real-time	Exon V of <i>ACTB</i> Gene	Act F: 5' GTSTGGATYGGHGGHTCBATC 3' Act R: 5' GAYTCRTCTAYTCCTSCCTTG 3' Act P: 5' ACCTTCCAGCAGATGTGGATC 3'	45	Piorkowski et al. 2014
<i>T. cruzi</i>	Conventional	Satellite-DNA	TCZ1: 5' CGAGCTCTTGCCACACGGGTGCT 3' TCZ2: 5' CCTCCAAGCAGCGGATAGTTCAGG 3'	40	Moser et al. 1989

extracted DNA from the 188 base pairs gel band using Zymoclean Gel DNA Recovery kit (Zymo Research, Irvine, California, USA), and sequenced using the amplification primers. There was a 100% match to *T. cruzi* (GenBank accession no. MF926630) shown by BLAST analysis (National Center for Biotechnology Information 2017).

This study represents the first time WTD have been identified as a potential reservoir species for *T. cruzi*. The WTD population is estimated to be around four million in Texas (Hall 2005). With such a large population of animals in the state, even a low prevalence could indicate a large number of animals are serving as reservoirs for this parasite. Our finding has important implications to the hunting community because there is a risk of transmission during the field dressing process. Garcia et al. (2015) reported that 93% (175/189) of Texas hunters field-dress carcasses directly after death, and 68% (94/139) reported they never wear gloves when field-dressing. If an animal is parasitemic, a hunter could be at risk of blood-borne infection if any abrasions or breaks in skin come in contact with infected blood.

Research is needed to further characterize the true prevalence of infection and burden of cardiac disease within sylvatic reservoir populations. Additionally, we need to better understand the risk this disease burden poses to populations with frequent wildlife contact. Without the necessary research to fully elucidate which wildlife is involved in propagating transmission of Chagas disease, we cannot adequately protect animal and human health. Hunters and other high-risk groups should be encouraged to use personal protective equipment when there is the potential for direct contact with blood.

We thank the staff of Wildlife Management Areas and Associations and hunters across the state who helped with specimen collections. We thank Micaela Sandoval for assisting with processing of specimens. This study was funded in part by an anonymous donor and the National Institutes of Health (project R21AI112647-01A1).

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