

Survey of Feral Swine (*Sus scrofa*) Infection with the Agent of Chagas Disease (*Trypanosoma cruzi*) in Texas, 2013–14

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ABSTRACT: Feral swine (*Sus scrofa*) are an invasive species and reservoir of numerous zoonotic pathogens in the US, and Texas leads the nation in the estimated population size of feral hogs. Texas also harbors enzootic transmission cycles of the protozoan parasite *Trypanosoma cruzi*, agent of Chagas disease. Given previous evidence that swine can serve as reservoirs of *T. cruzi* in Latin America and new evidence of triatomines (kissing bugs) feeding on swine in Texas, we measured the prevalence of *T. cruzi* infection in feral swine in Texas. From 2013 to 2014, we sampled blood and/or cardiac tissue from 78 feral swine across 14 Texas counties (seven with and seven without prior documentation of kissing bug occurrence) and used PCR and histopathology to detect *T. cruzi* infection. We determined an overall infection prevalence of 6% (3 of 54) based on PCR evaluation of cardiac tissue, and no blood samples were positive ($n=72$). All three positive pigs were from counties where kissing bugs are documented. No *T. cruzi* amastigotes were noted on histopathology ($n=54$). Sarcocysts were observed in 10 (18%) of the samples, five of which also had mild focal areas of degeneration and inflammatory cell infiltration. Eco-epidemiologic investigations can provide an assessment of contributions of feral hogs to maintenance of *T. cruzi* across a landscape to help protect human and animal health.

Key words: Chagas disease, feral swine, *Sus scrofa*, Texas, *Trypanosoma cruzi*.

Feral swine (*Sus scrofa*) are an invasive species in the US, with a population that has grown over the past several decades to exceed six million animals in 41 states (Bevins et al. 2014). Texas leads the nation in the estimated population size of feral hogs, with 1.8–3.4 million individuals. Despite management efforts that remove an estimated 29% of the population from Texas each year, this popu-

lation continues to grow (Timmons et al. 2012).

Feral swine have been implicated as reservoirs for at least 14 diseases transmissible to wildlife, domestic animals, and humans (Meng et al. 2009), but their involvement in the ecology of Chagas disease is uncertain. Chagas disease is a vector-borne cardiac disease of humans and animals caused by infection with the protozoan parasite *Trypanosoma cruzi*. Hematophagous triatomine (Hemiptera: Reduviidae: Triatominae) insects (also known as “kissing bugs”) serve as a vector for the parasite, which is transmitted through insect feces or consumption of an infected bug. Triatomines and *T. cruzi* are endemic across the southern US, and Texas is a hotspot for disease (Kjos et al. 2009; Tenney et al. 2014). Recent blood meal analyses of wild-caught kissing bugs in Texas identified pig DNA in a *T. cruzi*-positive kissing bug in Comal County (Kjos et al. 2013) and in a *Triatoma gerstaeckeri* from Gillespie County (S.A.H., R.C.-R. unpubl. data) that was submitted as part of an ongoing citizen science program (Curtis-Robles et al. 2015), confirming vector-host contact and raising questions about parasite transmission.

Laboratory challenge experiments and observations of natural infection suggest swine could contribute to the maintenance and transmission of *T. cruzi*. For example, domestic pigs inoculated with *T. cruzi* trypanosomes have been shown to develop parasitemia (e.g., Marsden et al. 1970). Studies of naturally infected pigs across Brazil (e.g., Herrera et al. 2008) and Mexico (e.g., Jiménez-Coello et al. 2012) have repeatedly shown *T. cruzi* infec-

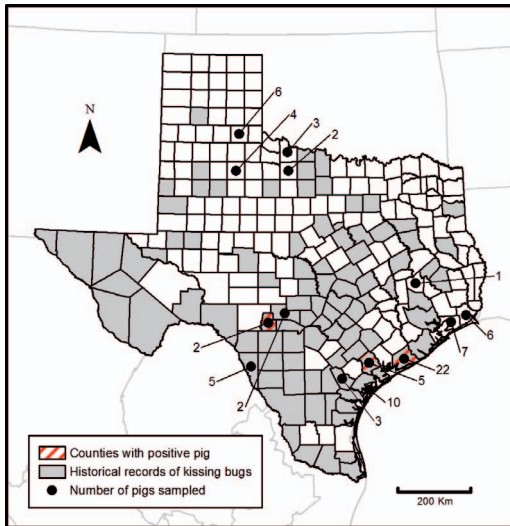


FIGURE 1. Geographic distribution of feral hogs (*Sus scrofa*) evaluated for infection with *Trypanosoma cruzi* in Texas, USA, 2013–14. Sample size per county is indicated; the three counties that each harbored one infected pig are in red. Gray shaded counties are those previously identified as having kissing bugs (Kjos et al. 2009).

tion or antibodies in pig blood using various methods. We measured the prevalence of *T. cruzi* infection in feral hog blood and heart tissue from diverse ecologic regions in Texas.

From June 2013 to June 2014, the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services harvested feral swine on wildlife management areas and ranches across Texas as part of ongoing management efforts. Fourteen counties were sampled (located between latitudes 28°07'N and 34°45'N and longitudes 93°50'N and 100°56'W), of which half had previous documentation of kissing bugs (Kjos et al. 2009; Fig. 1). In the field, postmortem blood samples and hearts were collected and stored at 4 C.

Hearts were dissected using a series of 2.5 cm thick, transverse cross sections and evaluated for gross abnormalities. A sample of right ventricle from each heart, and sections of tissue with apparent lesions, were fixed in 10% formalin for histopathologic evaluation. Samples (approximately 1 g) of right ventricle, apex, and clotted blood from

inside the heart chambers (when available) were removed for molecular analysis. Blood collected in the field was centrifuged, serum was removed, and approximately 250 μ L from the top of the clot was removed for molecular analysis. We performed DNA tissue extraction using an E.Z.N.A. kit (Omega Bio-Tek, Norcross, Georgia, USA).

To detect *T. cruzi*, we amplified satellite DNA using a Taqman qPCR reaction (Duffy et al. 2013). The DNA extracted from *T. cruzi* strain Sylvio X10 (American Type Culture Collection, Manassas, Virginia, USA) and DNA extracted from *T. cruzi*-positive kissing bugs collected in Texas (*Triatoma gerstaeckeri*, *Triatoma lecticularia*, and *Triatoma sanguisuga*) served as positive controls. Internal laboratory validations have defined cycle threshold values indicative of positive (<31), negative (>33), and equivocal (between 31 and 33) status. Equivocal samples were further tested using the *T. cruzi* 121/122 primers to amplify a 330 base pair region of kinetoplast DNA (Virreira et al. 2003). Histopathologic examination was performed at the Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas, US. Formalin-fixed samples were routinely processed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin.

From June 2013 to June 2014, we collected samples from 78 feral hogs in 14 Texas counties (Fig. 1), including matched whole blood and heart samples from 48 individuals, whole blood from 24 individuals, and whole heart from six individuals. Of the 54 hog hearts we tested using qPCR, three (6%) were infected with *T. cruzi*. The positive hogs were from Matagorda, Victoria, and Real counties in south and west Texas (Fig. 1) and were harvested in August 2013, September 2013, and June 2014, respectively. No blood samples were positive ($n=72$), including two from the same pigs with positive heart tissue.

No *T. cruzi* amastigotes were seen under histopathologic examination in the 54 sections of right ventricles examined. The majority of right ventricle samples (65%) had no significant lesions, including all three PCR-positive *T. cruzi*-infected individuals. Sarcocysts were

observed in 10 (18%) samples, five of which had mild focal areas of degeneration and inflammatory cell infiltration. For one of these five samples, the sarcocysts were apparently unassociated with the myocellular degeneration and inflammatory cell infiltration. Twelve additional histologic sections were prepared from tissue sections, including gross lesions identified during the dissection of the heart. On histopathologic examination, six had no significant microscopic lesions. Of the remaining six, five had quiescent sarcocysts present, and one had marked focally extensive cardiomyofiber loss and interstitial fibrosis, without an apparent cause.

Based on analysis of cardiac tissue the *T. cruzi* infection prevalence in feral hogs in Texas was 6% (3 of 54). All three positive pigs were harvested in counties where kissing bugs have been detected (Kjos et al. 2009). Over 35% of the hogs we sampled ($n=78$) were collected from counties with no prior documented kissing bugs populations (e.g., the Panhandle region, Fig. 1). Thus, a limitation of this study is that the infection prevalence we present is likely conservative with respect to geographic areas where kissing bugs are known to occur.

Although we detected *T. cruzi* DNA in cardiac tissue samples of three hogs using molecular methods, no blood samples were PCR positive, and histology did not reveal the organism in any sample. PCR has a high sensitivity and can detect *T. cruzi* even when levels of the parasite are low, such as in acute and chronic stages of disease (Gomes et al. 1999). However, in a dog model, duration of parasitemia was limited to a short window after initial infection (Barr et al. 1991), which limits the reliability of blood testing alone to detect infected individuals. Histopathology is highly specific for the detection of *T. cruzi* amastigotes, but the sensitivity is low, and previous investigators rarely detected parasites in heart tissue of chronically infected individuals (Palomino et al. 2000). In the apparent absence of amastigotes, however, we observed infiltrates of lymphocytes and plasma cells that may be associated with parasites not included in the sections.

Pigs are intermediate hosts for two species of sarcocysts, *Sarcocystis miescheriana* and *Sarcocystis suis hominis*; the latter is zoonotic (Dubey et al. 1989). In this study, we incidentally found sarcocysts in 10 of 54 (18%) right ventricle samples surveyed using histopathology, and additionally in five of 12 (42%) heart tissue sections that had gross abnormalities.

Feral hogs have the potential to connect sylvatic and domestic parasite transmission cycles due to their peridomestic residence. Given previous observations that kissing bugs feed on hogs (Kjos et al. 2013) and considering the opportunistic feeding behavior of feral hogs, which includes insectivory (Bevins et al. 2014), feral hogs are potentially exposed to *T. cruzi*. The nomadic behavior of feral hogs, however, may reduce their exposure to kissing bugs relative to nest-dwelling mammals, such as southern plains woodrats (*Neotoma micropus*), that are highly infected and contact kissing bugs that inhabit the nest environment (Charles et al. 2013). Given the increasing recognition of Texas as a hotspot for Chagas disease in recent years (Kjos et al. 2009; Tenney et al. 2014), contemporary information on potential wildlife reservoirs can be useful in providing an ecologic context for protecting human and animal health.

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