Epidemiology of *Trypanosoma cruzi* in Urban Dwelling Opossum (*Didelphis virginiana*) and Feral Cat (*Felis catus*) Populations of the Rio Grande Valley, TX

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Introduction

- Chagas disease is a zoonotic illness caused by a protozoan parasite, *Trypanosoma cruzi*, affecting an estimated 8 million people worldwide. The disease has multiple stages and can lead to life-threatening heart, digestive, and neurological issues.[1]

- *T. cruzi* is spread by triatomine vectors (Fig. 1) and maintained by diverse wildlife species across the Americas. The parasite may also be transmitted congenitally, via blood transfusion or organ transplant, and through ingestion of infected bugs or contaminated foods.[2] Oral transmission by ingestion of infected bugs is thought to be an important route of infection in sylvatic cycles.[3]

Figure 1. *Three species of triatomines (kissing bugs) that can be found in Texas. Left to right: Triatoma sanguisuga, Triatoma gerstaeckeri, Triatoma protracta (kissingbug.tamu.edu)*

- Despite well-recognized parasite transmission between wildlife and vectors in the southern US, the human and veterinary health burden of Chagas disease in the US is largely unknown. [4-6]

- In previous S. American studies, opossums have been noted as important reservoirs of *T. cruzi* and felines have shown to play an important role in the epidemiology of infection in triatomines. [7-8]

- In the Rio Grande Valley (RGV) of Texas where triatomine vectors are endemic, we recently described locally-acquired infections in humans and their pet dogs, and hypothesized that urban wildlife (opossums) and feral animals (domestic felines) serve as infectious reservoirs that bridge sylvatic and domestic transmission cycles.[9]

Figure 2. *The epidemiology of T. cruzi was studied in populations of urban dwelling opossums and feral felines from the Lower Rio Grande Valley of Texas (US-Mexico border)*

Objectives

1. Assess the prevalence of *T. cruzi* in urban dwelling opossums and the seroprevalence of feral feline populations of the Lower Rio Grande Valley

2. Identify the presence of *T. cruzi* DNA in various tissues collected from opossums and feral felines

3. Assess lesions in the heart and other tissues that may be attributed to *T. cruzi* infection via histopathology

Methods

- In 2017, we sampled urban-dwelling opossums (*Didelphis virginiana, n=100*) and feral cats (*Felis catus, n=167*) in the RGV.

Figure 3. *Opossums and a feline that were trapped by animal control in an urban location are awaiting evaluation in local animal shelter.*

- After euthanasia was performed by animal control for reasons unrelated to our study, whole blood, hearts, and other tissues were collected. Whole blood was centrifuged and separated into serum and clot.

- Feline serum samples were screened for *T. cruzi* antibodies using two independent rapid immunochromatographic tests (StatPAK & Chagas Detect Plus-InBios) designed for human use (Fig. 4), and an indirect fluorescent antibody (IgG) test provided by TVMDL.[9-10]

- Felines were considered antibody-positive if they were positive on at least two serological tests. Opossums were not subjected to serology due to incompatibility with tests.

Figure 4. *Examples of a StatPaK (left) & InBios (right) showing a clear control line and test line indicating the presence of T. cruzi antibodies*

- For a subset of felines, additional tissues were collected if they showed seropositivity on a StatPAK performed in the field. Similarly, additional tissues, such as intercostal muscle and anal gland tissue, were collected from a subset of opossums.

- DNA samples extracted from clot and tissues (E.Z.N.A. kit, Omega Bio-Tek, Norcross, GA) were amplified using Cruzi I/II/III probe-based qPCR to detect *T. cruzi* DNA. [5,12] Those with a Ct less than 40 were flagged as positive and subjected to discrete typing unit (DTU) determination using multiplex probe-based assay amplifying the spliced leader intergenic region (SL-IR). A sample was considered PCR positive (presence of parasite DNA) if a discrete typing unit was determined.

- Sections from each of the four chambers of the heart of selected animals, other PCR positive tissues, and a subset of negative tissues were processed routinely for histopathological assessment by a board-certified pathologist (CLH).

Results

- 22 felines (13.2%) were antibody-positive. Tissues in three felines (1.8%) were PCR positive and two of those felines tested positive on multiple tissues. (Table 1) No feline clot was PCR positive.

Table 1. Details of serology and tissue PCR for felines with at least one PCR positive tissue

Feline ID	StatPAK	InBios	IFA	PCR Positive Tissue	PCR Negative Tissue
F124	Negative	Negative	**Positive**	Esophagus Biceps femoris m. Sciatic n.	Clot, Cardiac m., Epicardial Adipose, Intercostal m., Mesentery, Colon
F129	**Positive**	Negative	Negative	Cardiac m.	Clot, Epicardial Adipose, Intercostal m., Mesentery, Colon, Trachea, Lung, Esophagus, Biceps femoris m.,Testes, Spleen, Splenic Adipose, Small Intestine, Liver
F133	Negative	**Positive**	**Positive**	Biceps femoris m. Sciatic n.	Clot, Cardiac m., Epicardial Adipose, Intercostal m., Mesentery, Colon, Esophagus

- Out of 100 opossums, 13 (13%) opossums tested PCR positive on at least 1 sample and 9 (9%) tested PCR positive on at least 2 samples. (Table 2) PCR positive tissues included clot (9%, n=100), heart (10%, n=100), anal gland secretion (6%, n=100), intercostal muscle (16.7%, n=42), and anal gland tissue (9.5%, n=42).

Table 2. Localization of *T. cruzi* DNA in various tissues of infected opossums

Opossum ID	Clot	Cardiac m.	Anal Gland Secretion	Intercostal m.	Anal Gland Tissue	Number of PCR Positive Tissues
OP07	**Positive**	**Positive**	**Positive**	N/A	N/A	3/3
OP09	Negative	Negative	**Positive**	N/A	N/A	1/3
OP16	Negative	Negative	**Positive**	N/A	N/A	1/3
OP17	**Positive**	**Positive**	Negative	N/A	N/A	2/3
OP34	Negative	Negative	**Positive**	N/A	N/A	1/3
OP63	**Positive**	**Positive**	Negative	**Positive**	Negative	3/5
OP69	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	5/5
OP72	**Positive**	**Positive**	Negative	**Positive**	**Positive**	4/5
OP81	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	5/5
OP82	Negative	**Positive**	Negative	Negative	Negative	1/5
OP84	**Positive**	**Positive**	Negative	**Positive**	Negative	3/5
OP91	**Positive**	**Positive**	Negative	**Positive**	**Positive**	4/5
OP94	**Positive**	**Positive**	Negative	**Positive**	Negative	3/5

- All PCR positive feline & opossum tissues (n=42) typed as TcI.

- The hearts of 2 infected opossums and 17 seropositive cats were examined histologically. (Fig. 5) The hearts of 5 of these cats exhibited minimal to moderate, multifocal, subacute lymphoplasmacytic inflammation. One opossum had mild, multifocal, subacute lymphoplasmacytic inflammation, and the other had moderate, multifocal, chronic lymphoplasmacytic myocarditis with myocyte loss and fibrosis in the left ventricle.

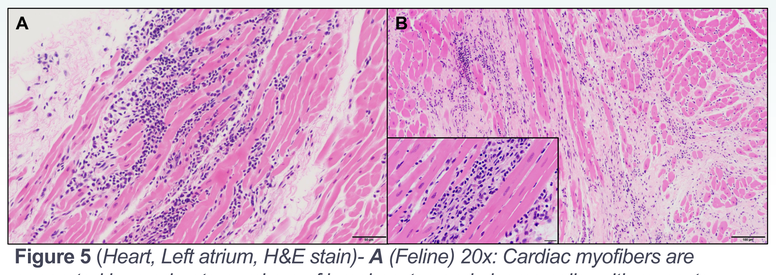

Figure 5 (*Heart, Left atrium, H&E stain*)- ***A*** *(Feline) 20x: Cardiac myofibers are separated by moderate numbers of lymphocytes and plasma cells, with myocyte degeneration and loss.* ***B*** *(Opossum) 10x: There is marked myocardial fiber loss with replacement fibrosis, with inflammation characterized by lymphocytes and plasma cells (inset 20x).*

Discussion

Despite both parasite DTUs TcI and TcIV being found in triatomine vectors in the region, the infected felines and opossums that were typed in this study were exclusively TcI-the DTU previously associated with human disease in the US. Only clot from opossums tested PCR positive for *T. cruzi,* indicating a greater potential of infectiousness to vectors than feral felines. Several of the cats and both opossums exhibited histopathologic changes consistent with those reported for *T. cruzi* infection in other species.[13] Our data implicates feral felines and opossums as wild reservoirs in an urban focus with ongoing autochthonous human and canine disease; these species must be considered in public health interventions.

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