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***Trypanosoma cruzi* and Incidental *Sarcocystis* spp. in Endangered Ocelots (*Leopardus pardalis*) of South Texas, USA**

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ABSTRACT: The federally endangered ocelot (*Leopardus pardalis*) population of south Texas, USA is declining; fewer than an estimated 80 ocelots remain. South Texas has robust transmission of *Trypanosoma cruzi*, the protozoan parasite causing Chagas disease in humans and various mammals. This parasite's impact in ocelots is unknown. Blood from live-trapped ocelots was collected by US Fish and Wildlife Service personnel in an annual monitoring program; additionally, tissues were obtained from carcasses collected from 2010 to 2017 around Laguna Atascosa National Wildlife Refuge in south Texas and placed in scientific collections. Variable samples were available from 21 ocelots: skeletal muscle ($n=15$), heart tissue ($n=5$), lung ($n=1$), kidney ($n=1$), spleen ($n=1$), liver ($n=1$), blood clot ($n=9$), and serum ($n=3$). Overall, 3/21 (14.3%) ocelots showed evidence of *T. cruzi* infection or exposure, with *T. cruzi* PCR-positive samples of skeletal muscle, heart, and blood clot, respectively. All three were infected with the *T. cruzi* discrete taxonomic unit "TcI"; one of these ocelots also had anti-*T. cruzi* antibodies. Lymphoplasmacytic inflammation was noted in the PCR-positive heart tissue and in some PCR-negative tissues from this and other individuals. Incidentally, *Sarcocystis* spp. were noted histologically in five ocelots. *Trypanosoma cruzi* infection and associated cardiac lesions suggest that this parasite should be further investigated in vulnerable populations.

Key words: *Leopardus pardalis*, ocelot, *Sarcocystis*, scientific collections, Texas, *Trypanosoma cruzi*.

The ocelot (*Leopardus pardalis*) is an elusive small felid with a geographic range from South America to Mexico and sparse areas of the southern US, specifically Texas and Arizona. Ocelots are listed as endangered by the US Fish and Wildlife Service. Historically in the US, ocelot populations extended

across eastern Texas and some southern and western parts of Arkansas and Louisiana (Haines et al. 2005). Currently, ocelots in Texas occur in small pockets of Tamaulipan thorn scrub habitat of south Texas on private ranches and on the Laguna Atascosa National Wildlife Refuge. Factors including habitat loss and vehicle collisions are drivers of population decline (Haines et al. 2006). Parasites may also play a role, but few studies have assessed their occurrence in south Texas ocelots (e.g., Pence et al. 2003; Haines et al. 2005).

Trypanosoma cruzi, the protozoan parasite causing Chagas disease, is a vector-borne parasite known to infect a wide range of mammals, including humans, domestic animals, and wildlife, in Texas (Curtis-Robles et al. 2016, 2017). Transmission occurs by contact with the feces of infected triatomine insect vectors. Additionally, oral transmission probably plays an important role when wild animals consume vectors or infected prey mammals (Hodo and Hamer 2017). In South America, *T. cruzi* is known to infect ocelots (Herrera et al. 2011; Rocha et al. 2013b). South Texas is an area with robust transmission of *T. cruzi*, and our previous studies highlighted high infection prevalence among triatomine vectors (>55%) in the region (Curtis-Robles et al. 2018). We used samples from live-trapped individuals and salvaged ocelots in scientific collections to assess *T. cruzi* infection and associated histologic lesions in south Texas ocelots.

From 2013 to 2017, blood samples were obtained from a subset of ocelots that were live-trapped during the US Fish and Wildlife

TABLE 1. Samples evaluated for *Trypanosoma cruzi* (PCR, histology, serology) from salvaged and live-captured ocelots (*Leopardus pardalis*) in south Texas, USA between 2010 and 2017.

USFWS ^a ocelot ID	TCWC ^a ocelot ID	Sex	Year of salvage or live capture	County of collection within Texas	Skeletal muscle, <i>T. cruzi</i> PCR ^b	Heart, <i>T. cruzi</i> PCR ^b	Clot, <i>T. cruzi</i> PCR ^b	<i>T. cruzi</i> serology ^b	Other samples available (all PCR negative)
OF225	65837	F	2012	Cameron	—	N/A	N/A	N/A	
OM279	65838	M	2014	Cameron	— ^c	N/A	N/A	N/A	
OMES5M	65839	M	2015	Willacy	— ^{c,d}	—	N/A	N/A	Lung, kidney, spleen, liver
OM314	65840	M	2016	Cameron	—	N/A	N/A	N/A	
OMES4F	65842	M	2015	Cameron	— ^c	N/A	N/A	N/A	
OM266	65843	M	2010	Cameron	—	N/A	N/A	N/A	
OM301	65844	M	2016	Cameron	—	N/A	—	—	
OM272	65845	M	2010	Cameron	+	N/A	N/A	N/A	
OAN	65846	M	2010	Willacy	—	N/A	N/A	N/A	
OM290	65847	M	2015	Cameron	— ^c	+ ^c	—	+	
OM292	65848	M	2015	Cameron	— ^d	— ^d	—	N/A	
OM267	65849	M	2010	Cameron	— ^c	N/A	N/A	N/A	
OM276	65850	M	2013	Cameron	— ^d	— ^c	N/A	N/A	
ESIM/Y7M	65851	M	2016	Willacy	— ^d	N/A	N/A	N/A	
OM263	65852	M	2017	Cameron	— ^{c,d}	— ^{c,d}	—	N/A	
OF265	N/A	F	2013 ^e	Cameron	N/A	N/A	N/A	—	
OM270	N/A	M	2013 ^e	Cameron	N/A	N/A	—	N/A	
OF274	N/A	F	2013 ^e	Cameron	N/A	N/A	—	N/A	
OM283	N/A	M	2015 ^e	Cameron	N/A	N/A	—	N/A	
OF304	N/A	F	2015 ^e	Cameron	N/A	N/A	+	N/A	
OF305	N/A	F	2015 ^e	Cameron	N/A	N/A	—	N/A	

^a USFWS = US Fish and Wildlife Service; TCWC = Biodiversity Research & Teaching Collections at Texas A&M University.

^b + = positive; — = negative; N/A = not applicable.

^c Lymphoplasmacytic inflammation observed (histology).

^d *Sarcocystis* spp. detected (histology).

^e Alive at time of sampling.

Service annual monitoring program. Additionally, we requested available ocelot tissue from salvaged animals that were maintained in the Biodiversity Research & Teaching Collections at Texas A&M University (TCWC) and the Gladys Porter Zoo. The salvaged ocelots in this study died primarily by vehicle collision from 2010 to 2017 around Laguna Atascosa National Wildlife Refuge. All samples were stored and frozen at -20°C prior to use in this study.

From 21 individuals, we opportunistically collected skeletal muscle ($n=15$), heart tissue ($n=5$), lung ($n=1$), kidney ($n=1$), spleen ($n=1$), liver ($n=1$), blood clot ($n=9$), and serum ($n=3$; Table 1). Six ocelots had more than one sample type (Table 1). Basic demographic

data (sex, year of collection, county records) were obtained from the collections record. For all tissues, sections were preserved in formalin, and additional sections were taken for molecular evaluation.

Tissues and blood clots from 20 ocelots were assessed molecularly for the presence of *T. cruzi* DNA. Following DNA extraction, we used a two-step process starting with a screening quantitative PCR to amplify a 166-base pair segment of the *T. cruzi* repetitive satellite DNA (Duffy et al. 2013). Any sample with a cycle threshold value less than 40 and a sigmoidal amplification curve was next subjected to discrete typing unit (DTU) determination using a quantitative PCR multiplex assay amplifying the *T. cruzi* spliced leader

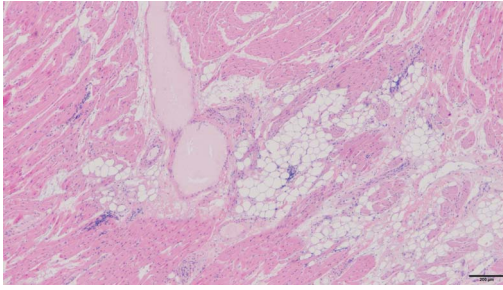


FIGURE 1. Histologic section of *Trypanosoma cruzi* PCR-positive heart of south Texas, USA, ocelot (*Leopardus pardalis*) OM290 showing lymphoplasmacytic myocarditis with myocyte degeneration and fatty degeneration. H&E stain.

intergenic region (Cura et al. 2015), which uses multiple probes, each specific for a DTU. Only samples that had positive results on both PCRs were considered to be infected. The formalin fixed tissues were routinely processed for histopathology, stained with H&E, and examined by a board-certified pathologist (C.L.H.).

All sera samples ($n=3$) were tested using a rapid immunochromatographic serologic test (Chagas Stat-Pak, Chembio, Medford, New York, USA). If the initial test was reactive, the sample was tested with the Chagas Detect Plus Rapid Test (InBios International, Inc., Seattle, Washington, USA). An ocelot was considered seropositive if both serologic tests produced positive results.

In total, 21 ocelots were represented in the study, with variable sample types available for each animal (Table 1). We discovered *T. cruzi* infection in three (14.3%, 95% confidence interval: 0.05–0.35) of 21 ocelots (Table 1), including PCR-positive muscle tissue, heart tissue, and clot samples from three different animals; all three tissues were infected with DTU TcI. The ocelot with a PCR-positive heart (OM290; TCWC 65847; Table 1) was the only ocelot (of three from which sera were tested) positive for anti-*T. cruzi* antibodies. This infection rate is very similar to that which we previously discovered in bobcats (*Lynx rufus*) of east-central Texas (14.3%; Curtis-Robles et al. 2016) and to the seroprevalence (11.4%) in stray domestic cats of south Texas

(Zecca et al. 2020b), both sympatric felid species. The ocelot with *T. cruzi* PCR-positive blood clot has been observed on game camera many times since the initial blood sample was collected, and it was seen with offspring in June 2020.

In the southern US, both TcI and TcIV are found in near-equal abundance in triatomines (Curtis-Robles et al. 2018). In Texas, strong associations between host species and DTU have been observed in raccoons (*Procyon lotor*; TcIV; Curtis-Robles et al. 2016), opossums (*Didelphis virginiana*; Zecca et al. 2020a), and feral cats (TcI; Zecca et al. 2020b). In Brazil, TcI has also been observed in ocelot blood clots from individuals from the same area where DTU TcII was observed in dogs (*Canis lupus familiaris*; Rocha et al. 2013a). Rodents are important reservoirs of *T. cruzi* (Hodo and Hamer 2017) and compose the majority of the ocelot diet in south Texas (Booth-Binczik et al. 2013). Rodents in a wildlife management area adjacent to habitat of south Texas ocelots have been found to be infected with *T. cruzi* of the same DTU (TcI) observed in our ocelots (Aleman et al. 2017), suggesting a possible route of oral transmission.

All tissues assessed histologically ($n=24$) were affected by moderate to advanced autolysis. We noted minimal to mild multifocal lymphoplasmacytic inflammation (myositis) in skeletal muscle of six animals that were PCR-negative for *T. cruzi*. In the skeletal muscle of two other PCR-negative animals, we saw focal and multifocal myodegeneration. Advanced autolysis prevented the histologic evaluation of the single PCR-positive skeletal muscle sample. Minimal to moderate multifocal lymphoplasmacytic inflammation was observed in hearts of three ocelots, including the animal with *T. cruzi* PCR-positive heart (OM290; TCWC 65847; Table 1). This animal showed moderate lymphoplasmacytic inflammation (myocarditis) with myocyte degeneration and fatty degeneration (Fig. 1). No significant lesions were observed in the lung, spleen, kidney, and liver samples. Lymphoplasmacytic inflammation in the heart (myocarditis) is the most common histologic lesion

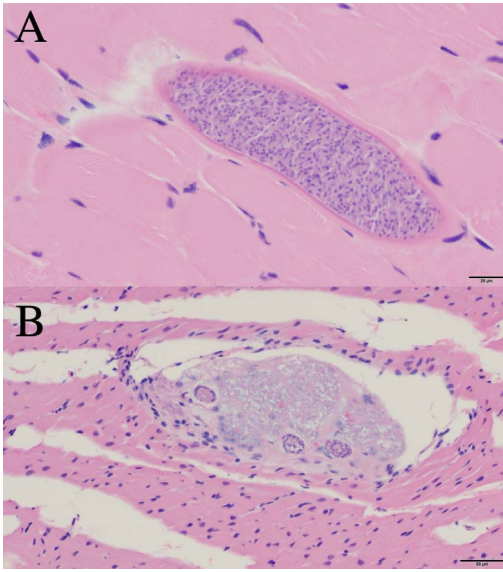


FIGURE 2. A. Histologic section of skeletal muscle of south Texas, USA, ocelot (*Leopardus pardalis*) OM263 with *Sarcocystis* sp. cyst filled with bradyzoites. H&E stain. B. Histologic section of heart of south Texas ocelot OM 292 with cluster of *Sarcocystis* sp. schizonts, showing minimal lymphocytic inflammation. H&E stain.

associated with chronic Chagas disease in humans and animals. However, this inflammation is not specific to *T. cruzi*; other pathogens, including bacteria (e.g., *Bartonella henselae*), viruses (e.g., feline panleukopenia virus, feline immunodeficiency virus), and parasites (e.g., *Dirofilaria immitis*, *Toxoplasma gondii*), could cause cardiac inflammation that appears similar (Zecca et al. 2020b). Alternatively, the inflammation could be due to *T. cruzi*, but the specific sections sampled for PCR may have had a level of infection that was below the detection threshold, given the conservative criteria used for positivity in this study.

Sarcocystis sp. schizonts or cysts with bradyzoites (sarcocysts) were observed incidentally in skeletal muscle of 5/15 (33.3%, 95% confidence interval: 0.15–0.58) examined ocelots (Fig. 2A). In muscle from two animals, degenerate *Sarcocystis* sp. schizonts were associated with mild lymphoplasmacytic inflammation. *Sarcocystis* spp. were also present in 2/5 examined hearts; these two animals also

showed sarcocysts in skeletal muscle. In one ocelot heart (OM292; TCWC 65848; Table 1), both sarcocysts and schizonts were observed, with minimal lymphocytic inflammation associated with the schizonts (Fig. 2B). The other heart (OM263; TCWC 65852; Table 1) contained a cluster of schizonts with no associated inflammation, and multifocal, mild, infiltrative lymphoplasmacytic inflammation and fibrosis not apparently associated with any schizonts.

A limitation to this study is the condition of the tissues that were collected from roadkill or had been frozen for many years. In addition to autolysis that hindered the histopathology examination, degraded DNA could result in false negatives. The level of infection presented here should therefore be interpreted as conservative. Further, ocelots with debilitating infections or severe disease would probably have been inactive and less likely to be trapped or killed out on the roads and may therefore not be represented in our sample.

The study of ocelots is challenging, as they are elusive cats that occur across both public and private lands. The partnership with scientific collections, as suggested by Cook and Light (2019), was essential in facilitating sample acquisition. Use of scientific collections, coupled with live animal surveillance and analysis of sympatric felid species, will advance the understanding of infectious diseases in ocelots.

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