VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 21, Number 10, 2021

DOI: 10.1089/vbz.2021.0019

Locally Transmitted *Trypanosoma cruzi* in a Domestic Llama (*Lama glama*) in a Rural Area of Greater New Orleans, Louisiana, USA

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Abstract

Trypanosoma cruzi-associated megaesophagus was diagnosed in a domestic Louisiana-born llama with no significant travel history. The llama resided in the same rural area of greater New Orleans, Louisiana, where the first human autochthonous case of Chagas disease was identified in the state. Venous blood from the llama tested positive for *T. cruzi* kinetoplastid DNA by conventional PCR. The cardiac evaluation was unremarkable, while thoracic radiographs revealed generalized megaesophagus. The llama received supportive care, but was ultimately humanely euthanized. The esophagus was severely distended throughout its length on necropsy, and histologic evaluation showed no microscopic changes in esophageal tissue and minimal to mild lymphoplasmacytic inflammation in cardiac tissue. *T. cruzi* DNA was detected by conventional PCR in the esophagus, small intestine, and blood despite no protozoan organisms being observed in multiple tissue sections examined. This report contributes to the growing body of evidence of local transmission of *T. cruzi* in the southern United States, and Chagas disease should be considered a differential diagnosis when evaluating llamas and other large animal species for esophageal dysfunction. There is little research describing megaesophagus or Chagas disease in llamas, and this report aims to increase awareness about this zoonotic disease that is becoming more frequently reported in the southern United States.

Keywords: Chagas disease, *Trypanosoma cruzi*, megaesophagus, esophageal dilatation, llama, autochthonous Chagas case

Introduction

Trypanosoma cruzi, the zoonotic parasite that causes Chagas disease, is a major public health concern in several countries spanning from Argentina to the southern United States (USA) (Bern et al. 2011). There is increasing evidence of local transmission of Chagas disease in the southern USA (Bern et al. 2011, Lynn et al. 2020). The first autochthonous human case in Louisiana was identified in June 2006 in a rural area of greater New Orleans (Dorn et al. 2007), and ~10% of all locally acquired cases in the USA since 2000 have originated from Louisiana (Dorn et al. 2007, LOPH 2018, Lynn et al. 2020). Human infection may occur secondary to spillover from zoonotic transmission cycles, and understanding the relationships between mammalian hosts, insect vector species, and the environment is critical to assess the risk of disease transmission in human and veterinary medicine.

Parasite transmission primarily occurs after feces of an infected, blood-sucking triatomine insect contaminate bite wounds or mucous membranes (Bern et al. 2011). There are

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more than 130 triatomine species in the Americas and while 11 have been reported in the USA, *Triatoma sanguisuga* is the main vector of *T. cruzi* in Louisiana. Initial surveys around the site of the first autochthonous human Chagas disease case reported positive infections in 56–66% of the *T. sanguisuga* insects collected (Dorn et al. 2007, Cesa et al. 2011, Herrera et al. 2015). The high prevalence of *T. cruzi* among this vector population is likely perpetuating local disease transmission among domestic and wild mammals in Louisiana's rural areas (Elmayan et al. 2019, Majeau et al. 2020).

T. cruzi has a complex life cycle that involves multiple vertebrate hosts in Louisiana and throughout the USA (Bern et al. 2011, Waleckx et al. 2014). In Louisiana, there is evidence of local transmission to wild mammals, including rodents (76%) (Herrera et al. 2015, Pronovost et al. 2018), opossums (37–60%) (Barr et al. 1991a, Houk et al. 2010), raccoons (23–43%) (Majeau et al. 2020), and armadillos (1–37%) (Yaeger 1988, Barr et al. 1991a). Additionally, the reported prevalence among domestic dogs in Louisiana has ranged from 1% to 16% over the past 40 years (Elmayan et al. 2019), and the overall prevalence among nonhuman primates born and raised at the Tulane National Primate Research Center in Covington, Louisiana, was 1.6% (Dorn et al. 2012, Herrera et al. 2019).

While it is well known that triatomine vectors are nonspecific feeders and will feed on livestock and other large animals (Kjos et al. 2013, Dumonteil et al. 2018, 2020), there are no reports of Chagas disease in llamas or other camelids, and the clinical features of the disease in these species remain unclear. In humans, *T. cruzi* manifests in acute or chronic forms of the disease (WHO 2015). Chronic Chagas disease is characterized by *T. cruzi* amastigotes becoming localized in particular tissues and organs. Cardiac and digestive tissues are most commonly targeted, which may lead to arrhythmias and/or heart failure and megaesophagus/megacolon, respectively (WHO 2015). Specific clinical features in animals have been documented in mice (Okumura and Correa Neto 1961), dogs (Okumura and Correa Neto 1961, Barr et al. 1991b), and nonhuman primates (Marsden et al. 1976, Mubiru et al. 2014), and cardiac and gastrointestinal tissues were similarly affected. Presented here is the first documented case of possible *T. cruzi*-associated megaesophagus in a domestic llama (*Lama glama*) located in the same geographic area as the first human autochthonous Chagas case (Dorn et al. 2007), as well as specific clinical features of the disease and diagnostic methods used.

Materials and Methods

Clinical evaluation for Chagas disease

In 2017, we received the case of a 4-year-old, male castrated llama; born near Covington, Louisiana, USA; raised in the greater New Orleans area; and with no travel history. The llama was experiencing significant weight loss and choking episodes from the time of his castration in March 2016. Episodes were characterized by a rolling motion in the midsection of his neck with a grunting noise, potentially indicating an inability to appropriately regurgitate food. Intermittent, mild upper respiratory infections were also reported. To manage the weight loss and suspected megaesophagus, the llama was transitioned to elevated feeding, soaking of feed, monthly ivermectin deworming, fenbendazole deworming every 3–6 months, and daily administration of Red Cell vitamin–iron–mineral supplement. The llama lived in an area where zoonotic transmission of *T. cruzi* had historically been documented (Dorn et al. 2007), and based on the similar clinical presentation to chronic human Chagas disease, a jugular venous blood sample was collected and evaluated for *T. cruzi* infection.

The llama was evaluated at the Louisiana State University Veterinary Teaching Hospital (LSU VTH) and a comprehensive physical examination, oral examination, complete blood count (CBC), serum biochemical assay, and McMaster's fecal flotation were performed. After continued decline in quality of life, the llama returned to the LSU VTH for an echocardiogram, electrocardiogram (ECG), radiographs, and humane euthanasia with postmortem examination.

Pathological and histological evaluation

Following euthanasia, a complete necropsy was performed, and all tissues were evaluated macroscopically. Routine tissue collection was performed according to standard procedures. Tissue samples were fixed in 10% buffered formalin solution, trimmed, and embedded in paraffin blocks. Five-micrometer sections were prepared and stained with hematoxylin and eosin (H&E) for routine histopathological evaluation. Additional tissues from the heart (atria, ventricles, septum, and apex), pericardial adipose, esophagus, small intestine (pooled duodenum, jejunum, and ileum), descending colon, liver, kidney, spleen, mesenteric lymph node, and skeletal muscle (right and left thighs) were collected for DNA extraction.

Molecular identification of T. cruzi

Blood samples, collected in 2017 and 2019, and necropsy tissues, including the esophagus, heart, and small intestine, were analyzed for the presence of *T. cruzi* DNA. Blood samples were mixed with an equal volume of 6 M guanidine-HCL and 0.2 M EDTA (pH 8) and stored at room temperature (Elmayan et al. 2019), and tissues were stored at 4°C. DNA from blood and tissue samples was promptly extracted using the DNeasy extraction kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions and stored at 4°C. Remaining blood-guanidine samples were kept at room temperature and tissues were kept at −20°C for long-term storage. Conventional PCR was performed on all samples using two different molecular markers for *T. cruzi*. TCZ1 (5′CGAGCTCTTGCCCACACGGGTGCT-3′) and TCZ2 (5′-CCTCCAAGCAGCGGATAGTTCAGG-3′) primers were used to target satellite DNA (satDNA) (Moser et al. 1989) that produce a band of 188 bp specific for *T. cruzi*, and the minicircle variable region of the kinetoplast DNA (kDNA) was targeted using the primers, 121 (5′-AAATAATGTACG GG(T/G)GAGATGCATGA-3′) and 122 (5′-GGTTCGATT GG GGTTGGTGTAATATA-3′), amplifying a band of 330 bp specific for *T. cruzi* kDNA (Wincker et al. 1994). DNA from a *T. cruzi* reference strain WB1 was used as the positive control. Molecular grade water was also used as the negative control in the PCR. The PCR products were separated on a 2% agarose gel and visualized using a Bio-Rad Gel Doc system (Bio-Rad Laboratories, Hercules, CA).

Results

Clinical evaluation

The llama had a poor body condition with a weight of 63.6 kg (previously recorded 72.5 kg in 2016), continued difficulty regurgitating food, and frequent sneezing of C1 compartment contents from nasal passages. CBC and serum chemistry values are provided in Table 1. The echocardiogram revealed normal structure, motion, and position of the cardiac chambers. No evidence of valvular incompetence or congenital malformations was noted. Limited information is available regarding normal cardiac dimensions of new-world camelids, but patient dimensions were consistently lower than published measurements of cardiac dimensions for adult Huacaya alpacas (72 ± 6 kg) (Margiocco et al. 2009). The electrocardiogram revealed a normal sinus rhythm.

Table 1. Serum Biochemical Analysis and Complete Blood Cell Count

Parameter[a]	*Result*[b]	*Range*[c]	*Units*
Chemistry panel			
Glucose	138	71–149	mg/dL
AST	**120**	**36–76**	**U/L**
GGT	**39**	**6–29**	**U/L**
Alk phosphatase	**71**	**0–10**	**U/L**
CK	37	14–238	U/L
TBIL	0.1	0.0–0.2	mg/dL
Total protein	5.9	4.7–7.3	g/dL
Albumin	3.7	3.6–6.1	g/dL
Globulin	2.2		g/dL
BUN	14	9–34	mg/dL
Creatinine	2.76	1.1–3.2	mg/dL
Ca	8.7	7.9–10.9	mg/dL
Phos	3.1	2.6–10.7	mg/dL
Na	148	148–158	mM
K	4.5	3.7–6.2	mM
Cl	118	103–125	mM
Bicarbonate	21.0		mM
AGAP	13.5	13–27	mM
MG	2.1		mg/dL
Calculated OSMO	296		mM
CBC			
RBC	11.58	10.3–15.2	$10^6/\mu L$
HGB	13.4	10.6–16.5	g/dL
Platelet count	**282**	**299–775**	**$10^3/\mu L$**
MPV	**7.1**	**4.0–6.5**	**fL**
PCT	0.2		%
PLT comment	With clumps		
Plasma appearance	Normal		
Protein	6.4	5.3–7.0	g/dL
PCV	30		%
HPP	300		mg/dL
Tech:	513		
WBC	8.8	7.89–20.27	$10^3/\mu L$
Neutrophils	5.5 (62)	3.67–10.96	$10^3/\mu L$ (%)
Lymphocytes	1.58 (18)	2.13–6.41	$10^3/\mu L$ (%)
Monocytes	0.1 (1.0)	0.08–0.71	$10^3/\mu L$ (%)
Eosinophils	1.7 (19.0)	0.16–3.29	$10^3/\mu L$ (%)

[a]Values outside the reference range are listed in bold letters.
[b]Reference values provided by UC Davis School of Veterinary Medicine, Davis, CA.
[c]Values provided by LSU VTH&C Clinical Pathology Laboratory, Baton Rouge, LA.

Thoracic radiographs showed generalized megaesophagus, which was most severe in the thoracic esophagus (Fig. 1a). Gas was present in the cranial cervical esophagus, and there was gas and fluid in the caudal cervical and thoracic esophagus, creating a fluid line, with a small amount of gravity-dependent mineral debris. Mild alveolar opacity was noted in the right ventral lung, which was thought to be due to either artifacts from superimposed thoracic limbs or potential aspiration pneumonia. Normal ingesta and mineralized material were noted in C1 and C3 of the stomach; the remainder of the examination was normal.

Macroscopic findings. Necropsy identified severe megaesophagus as the primary gross finding (Fig. 1b). The esophagus was distended throughout its length, with narrowing in a focal area of the thoracic segment associated with the overlying azygos vein where it coursed over the esophagus. The esophageal measurements were as follows: cervical/neck portion, 2.5 cm diameter; middle neck portion, 1.2 cm diameter; thoracic inlet portion, 2.5 cm diameter; intrathoracic portion (cranial to the azygos vein), 5.5 cm diameter; caudal to the azygos vein, 6.5 cm diameter; and at the esophageal hiatus, 5 cm diameter (reference range: 2.54 cm cranial cervical; 3.89 cm caudal thoracic (Sukon et al. 2009)). No significant lesions and discoloration of the heart were noted. The right ventricular wall (RV) was 0.5 cm thick; the left ventricular wall (LV) and interventricular septum measured 1.7 cm for an RV:LV ratio = 1: 3.4 (reference range: RV:LV = 1: 2–3). Rare individual saccules within the C1 stomach compartment contained concretions and were considered to be within normal limits. No other significant lesions were noted.

Histologic findings. Histologic examination of the heart showed minimal to mild alterations, including occasional rowing of myocardial nuclei and subjectively increased numbers of sarcolemmal nuclei and interstitial cells. The interventricular septum showed similar alterations as well as a focal area degenerative cardiomyocytes characterized by shrunken and hypereosinophilic cytoplasm and pyknotic nuclei surrounded by a few mononuclear cells. The left heart showed a single aggregate of lymphocytes and plasma cells within the interstitium (Fig. 2). Sections of skeletal muscle from both right and left thighs showed similar minimal to mild alterations, including subjectively increased numbers and occasional rowing of sarcolemmal nuclei and mildly swollen sarcoplasm within individual myofibers.

There were no histologic abnormalities identified in six sections of the esophagus. The bronchioles and alveoli contained scattered protozoan organisms, but were considered incidental findings (terminal aspiration). The stomach contained cross sections of nematode larvae, which were considered to be insignificant in this llama. Parasitological testing yielded Trichostrongylus-type ova and Eimeria spp., which were also not considered significant. The mucosal capillaries of the small and large intestines were congested, and lymphoid follicles of the mesenteric lymph node were hyperplastic. No significant abnormalities were seen within the examined sections of the spleen, liver, kidney, tongue, and urinary bladder. No protozoan organisms compatible with *T. cruzi* were observed in any tissues.

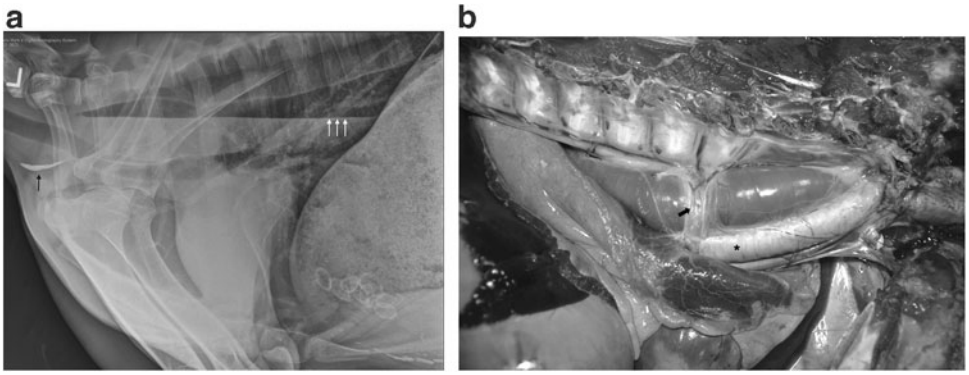

FIG. 1. Megaesophagus. **(a)** *Left* lateral thoracic radiograph showing diffuse distention of the thoracic esophagus. The esophagus contains fluid and gas that creates a gas–liquid interface (*white arrows*) as well as gravity-dependent mineral debris (*black arrow*). **(b)** Gross necropsy image of the esophagus, located between the vertebral *column* dorsally (*top* of photo) and the trachea ventrally (*asterisk*), enlarged, and focally narrowed by the overlying azygos vein (*arrow*) where it courses over the esophagus.

Molecular identification of T. cruzi

The presence of *T. cruzi* DNA was successfully identified in blood collected in 2017 (Fig. 3a), blood collected in 2019 (Fig. 3b), and in tissues from the esophagus and small intestine using kDNA as the molecular target (Fig. 3b). All other tissues, including cardiac tissues, showed no *T. cruzi* DNA amplification targeting kDNA. There was no *T. cruzi* DNA amplification using the satDNA as the molecular target, and positive and negative controls performed as expected for both reactions.

Discussion

The clinical evaluation and molecular diagnostics observed in the present case are consistent with *T. cruzi*-associated megaesophagus. To the best of our knowledge, this is possibly the first case of a chagasic mega syndrome documented in a llama or any other large animal species, as it has previously only been described in nonhuman primates (Marsden et al. 1976) and experimental dogs and mice (Okumura and Correa Neto 1961). Although megaesophagus is not uncommon in camelids (Watrous et al. 1995), there are very few cases documenting such findings. Esophageal dysfunction secondary to congenital vascular ring abnormalities has been reported in crias (McKenzie et al. 2010), and among llamas, idiopathic cases of acquired megaesophagus were most frequently reported (Watrous et al. 1995). There have been no previous reports of acquired megaesophagus associated with infectious diseases in llamas, although myasthenia gravis and generalized inflammatory myopathies, including those resulting from infectious disease, are common neuromuscular disorders that cause acquired megaesophagus in small animals (Evans et al. 2004, Mace et al. 2012). Tetanus produces esophageal dysfunction in humans and dogs (Dieringer and Wolf 1991) and *Toxoplasma gondii*, *Neospora caninum*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Rickettsia rickettsii* have also been implicated in megaesophagus (Mace et al. 2012). However, the history, symptoms, detection of *T. cruzi* DNA, and mild histologic changes are consistent with a clinical diagnosis of *T. cruzi* and potentially associated megaesophagus. Additionally, *T. cruzi* DNA was also found in small intestine tissue, showing a possible case of digestive Chagas disease (WHO 2015).

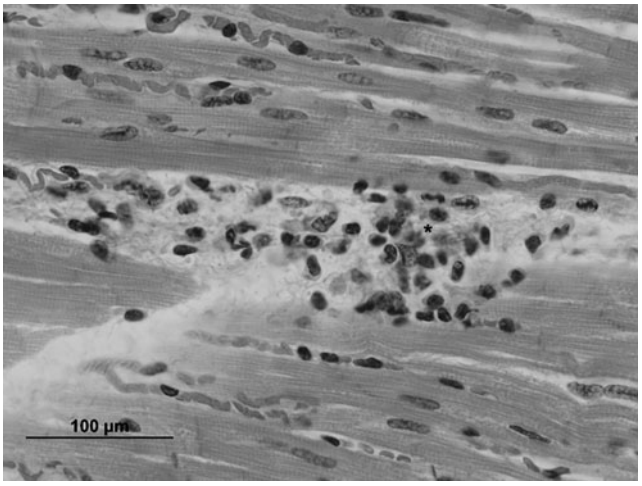

FIG. 2. Photomicrograph of the heart with a small area of lymphocytic to plasmacytic inflammation (*asterisk*) in the interstitium, H&E stain (scale bar = 100 μm).

These findings are consistent with those described in humans and some veterinary species. In humans, dilation and thickening of the esophagus result from the presence of inflammatory lesions within layers of smooth muscle cells and subsequent parasympathetic denervation (Teixeira et al. 2006), which can occur throughout the digestive tract, but are generally more common in the esophagus and colon (Teixeira et al. 2006). Nonetheless, it has been reported that inflammatory lesions were present in only half of human *T. cruzi* megaesophagus cases despite positive identification of parasite DNA by PCR in 77% of esophageal tissues and 90% of blood samples (Lages-Silva et al. 2001). This tissue tropism of the parasite was reflected in the current case as DNA was positively identified in the esophagus and small intestine and neither organ showed histologic changes or signs of inflammatory infiltrates. *T. cruzi* lesions are commonly unevenly distributed across affected tissues and organs (Teixeira et al. 2011), which possibly contributed to the apparent absence of histologic lesions in this llama's

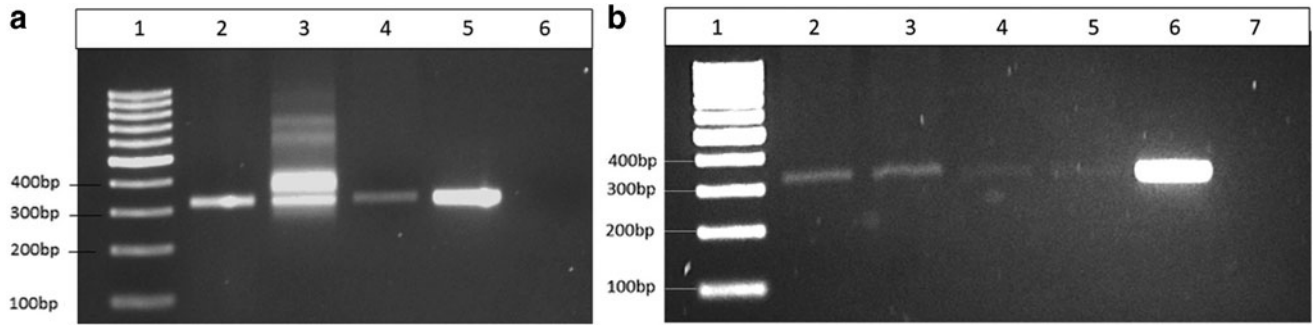

FIG. 3. DNA gel electrophoresis of PCR amplicons from llama blood and tissue samples from different collection years. **(a)** Blood samples collected in the year 2017: Lane 1: 1-kb ladder; Lane 2: blood sample No. 1 (9/11/17); Lane 3: blood sample No. 2 (9/27/2017); Lane 4: blood sample No. 3 (10/2/2017); Lane 5: *Trypanosoma cruzi* positive control (strain WB1); and Lane 6: negative control. **(b)** Blood and tissue samples collected in the year 2019: Lane 1: 1-kb ladder; Lane 2: blood sample No. 1 (9/13/2019); Lane 3: blood sample No. 2 (10/28/2019); Lane 4: esophagus tissue; Lane 5: small intestine tissue; Lane 6: *T. cruzi* positive control (strain WB1); and Lane 7: negative control.

esophagus. This, in addition to blood samples that tested positive for *T. cruzi* DNA, supports the diagnosis of Chagas disease and associated megaesophagus.

A small population of lymphocytes and plasma cells was identified in the left heart, consistent with very common findings of lymphoplasmacytic myocarditis in humans (Higuchi Mde et al. 1993, Teixeira et al. 2006), as well as chagasic dogs (Barr et al. 1991b), coyotes (Curtis-Robles et al. 2016, Hodo et al. 2020), raccoons (Curtis-Robles et al. 2016), and cats (Zecca et al. 2020). Enlargement of the heart is a common feature of Chagas disease in humans (Teixeira et al. 2006), and it is unclear if the increased RV:LV ratio is associated with *T. cruzi* infection. Alterations in tissue architecture and cardiac function due to Chagas disease result from the presence of inflammatory infiltrates within the heart tissue and subsequent damage to cardiomyocytes and the cardiac conduction system (Teixeira et al. 2006). The severity of tissue damage correlates with the degree of inflammatory infiltration, and *T. cruzi* parasites are rarely found microscopically (Higuchi Mde et al. 1993). Similar alterations may occur in striated skeletal muscle, and all observed changes within this llama's heart and skeletal muscle were minimal to mild and scattered. These changes could be suggestive of prior cardiac or muscular damage with regenerative change, although such damage could be nonspecific for Chagas disease. Even so, the mild histologic findings, the limited presence of degenerative cardiomyocytes and lymphocytic to plasmacytic inflammation, and the absence of parasitic organisms adequately correspond to the normal echocardiogram and ECG findings observed upon examination.

There is limited information available detailing how Chagas disease manifests in large mammals and what clinical presentations are more common. One report on experimental pigs showed no macroscopic alterations from *T. cruzi*, yet demonstrated histologic perivasculitis in the heart, meninges, and kidney, as well as lymphoid hyperplasia with necrosis in the spleen (Yauri et al. 2016). An additional report described symptoms and pathologic lesions observed in a chagasic horse that were primarily neurologic, and no histologic lesions were apparent in the heart or other organs (Bryan et al. 2016). The horse and the llama presented here received advanced diagnostics as companion animals, but in contrast, most food animal species do not live long enough for chronic signs of Chagas disease to develop or are sent to slaughter if signs of acute illness are shown. These practices and the likelihood that advanced diagnostics may be cost-prohibitive for large animals kept as companion animals may contribute to the lack of awareness and underreporting of this parasite in large animal species.

Human infections with *T. cruzi* are treated with the antitrypanosomal medications, benznidazole and nifurtimox (Rodriques Coura and de Castro 2002, Bern et al. 2007). Recently, both drugs were approved by the U.S. Food and Drug Administration (FDA) for the treatment of Chagas disease in pediatric patients under 18 years of age. (Traynor 2017, Thakare et al. 2021). Effective treatment has been recorded in acute phases and recent chronic infections, and it is recommended that patients receive treatment for late chronic infections without clinical manifestations or if clinical changes are mild (Rodriques Coura and de Castro 2002). These drugs have been rarely used in canine Chagas cases as supportive care is more common (Guedes et al. 2002, Bern et al. 2011). Similar to humans, these medications are more likely to be effective in earlier phases of the disease in the absence of more severe disease pathology (Guedes et al. 2002). It is unclear if these drugs would be effective against *T. cruzi* in camelids, but treatment would have likely been unrewarding in this case due to the severity of megaesophagus.

The prognosis for *T. cruzi*-associated megaesophagus in ruminants and camelids is likely poor, even with aggressive medical management. The ability of these animals to adequately digest and obtain nutrients from feed and forage high in cellulose is possible through a combination of physiologic factors. Salivation, chewing, swallowing, regurgitation, and rechewing are essential for adequate nutrient digestion in all ruminants and camelids, including llamas (Esteban and Thompson 1988, Church 1993). C1 and C2 of the camelid stomach, equivalent to the rumen and reticulum of a ruminant (Esteban and Thompson 1988), house a population of microorganisms that digest and break down plant material and produce volatile fatty acids, which are essential for energy production (Church 1993). The esophagus plays a critical role in ensuring the bidirectional movement of food for chewing and rechewing (Church 1993) and the presence of megaesophagus can hinder this process. This was observed in the case reported here and what ultimately contributed to the llama's decline in health and eventual euthanasia.

This case provides a detailed clinical evaluation of locally transmitted Chagas disease in a new mammalian host and also

contributes to the growing body of evidence that *T. cruzi* is actively circulating in humans and animals within the USA. It is critical to fully describe all *T. cruzi* infections in any chagasic mammalian hosts to understand what transmission cycles may exist among triatomines, humans, domestic species, and local wildlife. These data will help bridge knowledge gaps regarding the eco-epidemiology of the parasite, which is essential for estimating the subsequent risk of disease in veterinary medicine and the potential for spillover into human medicine (Pronovost et al. 2018). It is feasible that disease transmission has persisted among humans and animals throughout the previous decade in this rural area of greater New Orleans as this llama, born in the USA, had a limited travel history and resided in the same geographic area where *T. cruzi* has historically been detected over several years. As such, it is probable that the prevalence of disease in human and veterinary medicine is underreported and there is an increased need for proactive surveillance.

Conclusion

This report describes the first documented case of locally transmitted Chagas disease and associated megaesophagus in a domestic llama. There is little information available detailing Chagas disease or megaesophagus in llamas, and this report increases awareness for owners and veterinarians about this zoonotic parasite and provides veterinarians new diagnostic information for the clinical setting. This information collectively contributes to characterization of *T. cruzi* in the southern USA, yet there are still many features of local disease transmission that remain unclear.

Ethics Statement

All procedures were performed during the standard clinical care of the animal, with consent from the owner. The animal was treated humanely and received a high standard of veterinary care at all times.

Authors' Contributions

C.P.H. and E.D. conceived the study and designed the study protocol. C.A.H., C.M.S., and R.E.B. collected blood samples and clinically evaluated the case. R.J. interpreted diagnostic imaging. A.M.C. performed the cardiac evaluation. E.S., R.W.B., J.M.T., and C.A.H. performed necropsies and provided tissue samples. E.S. and R.W.B. performed and interpreted histopathologic examination of tissues. A.M., H.P., and J.M.T. carried out the molecular analysis. C.P.H., E.D., and J.M.T. carried out interpretation of the data. All authors drafted the manuscript and critically revised it for intellectual content. All authors read and approved the final manuscript. C.P.H. and E.D. are guarantors of the article.

Acknowledgment

The authors would like to thank Weihong Tu for her assistance with molecular diagnostics.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This work was funded, in part, by the Tulane ByWater Institute Faculty Fellowships in Interdisciplinary Collaboration 2020 to C.P.H., the Louisiana Board of Regents through the Board of Regents Support Fund [contract number LE-SASF (2018–21)-RD-A-19] to E.D., and the Ruth L. Kirschstein National Research Service Award [grant number 5T32OD11124–15 (MPI)] to J.M.T.

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