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Short communication

Chagas disease in a Texan horse with neurologic deficits

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ABSTRACT

A 10-year-old Quarter Horse gelding presented to the Texas A&M University Veterinary Teaching Hospital with a six month-history of ataxia and lameness in the hind limbs. The horse was treated presumptively for equine protozoal myeloencephalitis (EPM) based on clinical signs but was ultimately euthanized after its condition worsened. Gross lesions were limited to a small area of reddening in the gray matter of the thoracic spinal cord. Histologically, trypanosome amastigotes morphologically similar to *Trypanosoma cruzi*, the agent of Chagas disease in humans and dogs, were sporadically detected within segments of the thoracic spinal cord surrounded by mild lymphoplasmacytic inflammation. Ancillary testing for *Sarcocystis neurona*, *Neospora* spp., *Toxoplasma gondii* and *Leishmania* spp. was negative. Conventional and real time polymerase chain reaction (PCR) of affected paraffin embedded spinal cord were positive for *T. cruzi*, and sequencing of the amplified *T. cruzi* satellite DNA PCR fragment from the horse was homologous with various clones of *T. cruzi* in GenBank. While canine Chagas disease cases have been widely reported in southern Texas, this is the first report of clinical *T. cruzi* infection in an equid with demonstrable amastigotes in the spinal cord. In contrast to previous instances of Chagas disease in the central nervous system (CNS) of dogs and humans, no inflammation or *T. cruzi* amastigotes were detected in the heart of the horse. Based on clinical signs, there is a potential for misdiagnosis of Chagas disease with other infectious diseases that affect the equine CNS. *T. cruzi* should be considered as a differential diagnosis in horses with neurologic clinical signs and histologic evidence of meningomyelitis that originate in areas where Chagas disease is present. The prevalence of *T. cruzi* in horses and the role of equids in the parasite life cycle require further study.

1. Introduction

Chagas disease, or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi* (Gutiérrez et al., 2013; Kjos et al., 2008). It is endemic in many non-Caribbean Latin American countries with a purported 5.7 million people currently infected (Anon, 2010). The protozoan multiplies within arthropod vectors of the Reduviidae family (*Rhodnius* spp., *Panstrongylus* spp. and *Triatoma* spp.), also known as cone-nose or kissing bugs, which harbor the protozoan epimastigote and metacyclic trypomastigote life stages within the midgut and hindgut, respectively (Gutiérrez et al., 2013). Triatomine bugs transmit *T. cruzi* by defecating infectious metacyclic trypomastigotes around cutaneous bite wounds or mucous membranes, thereby allowing access to the host's blood stream where the trypomastigotes can either infect host cells to form amastigotes or infect other kissing bugs feeding on the host (Gutiérrez et al., 2013). Humans can become infected transplacentally, via blood or organs through transplantation (Rassi et al., 2012). While autochthonous cases of acute Chagas disease in people remain rare in the United States (US), there are an estimated 300,000 chronic cases in immigrants from endemic countries living in the US (Bern and Montgomery, 2009). Chagas disease has been frequently reported in dogs from the southeastern US where *T. cruzi* persists through a sylvatic life cycle that involves infection of raccoons, opossums, armadillos, and skunks (Bern et al., 2011). While the arthropod vector will readily feed on horses and other live-

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stock in experimental situations (Grundemann, 1947), the various life stages of *T. cruzi* have never been reported before in peripheral blood or tissues from horses.

2. Case report

A 10-year-old, Quarter Horse gelding, used for barrel racing, presented in mid-summer to the Texas A&M University Veterinary Medical Teaching Hospital with a six-month history of lameness and ataxia in the hind limbs. The horse lived in southern Bexar County, Texas and had been previously diagnosed with chronic impar ligament desmitis in the right front foot. Four months prior to referral, the horse was seen by a veterinarian when the owner noted a new left hind limb lameness. At that time, the gelding was ataxic in both hind limbs, with the right hind more severely affected than the left hind. Cerebrospinal fluid (CSF) was tested for antibodies against *Sarcocystis neurona*, the causative agent of equine protozoal myeloencephalitis (EPM), via surface antigen 2, 4/3 enzyme-linked immunosorbent assay (ELISA), Western Blot and indirect fluorescent antibody test (IFAT). All tests on the CSF were negative for *S. neurona,* and a *Neospora hughesi* IFAT was also negative. However, due to the difficulty of ante-mortem diagnosis of EPM in horses, treatment with ponazuril (5 mg/kg, per os) for 28 days was initiated. An additional 10-day course of levamisole and tapering course of dexamethasone (starting at 0.1 mg/kg) were administered after the horse failed to improve with ponazuril treatment. Nuclear scintigraphy (bone scan) did not reveal any significant musculoskeletal abnormalities.

Upon neurologic examination at referral, no cranial nerve deficits were noted and mentation was appropriate. A standing musculoskeletal exam was unremarkable and no muscle atrophy was appreciated. Range of motion of the cervical vertebrae was within normal limits and the cutaneous trunci reflex was intact. During a moving examination, the patient had difficulty pivoting on his hind limbs and would swing his rear legs into wide arcs (circumduction) when circled tightly. The horse also had difficulty navigating around low obstacles, such as curb stones, and would scrape the tops of his hind hooves on the pavement (knuckling) when transitioning from a walk to a trot. The hind end deficits were consistent with upper motor neuron paresis, as on tail pull the gelding had good resting muscle tone with adequate extensor strength while standing, but was easily pulled to the side while walking. His ataxia was asymmetric, with the right side more severely affected than the left, and he was assessed as Grade 3/5 and 4/5 ataxic in the left and right hind limbs, respectively. Assessment of the fore limbs was complicated by the severity of ataxia in the hind limbs, but they were apparently unaffected. Lesion localization was presumed to be within the T3-L3 region based on neurologic examination. Radiographs of the cervical and lumbar vertebrae revealed no abnormalities. Ultrasonography of the caudal lumbar vertebrae, sacrum and pelvis (both transcutaneous and transrectal) was within normal limits. Serum vitamin E concentration was within the normal range (3.32 μg/mL; reference 2.5–4.0 μg/mL) and there were no hematologic or serum chemistry abnormalities. Euthanasia was elected three months after neurologic evaluation due to worsening condition and poor prognosis for recovery.

A necropsy was conducted at the Texas A&M Pathology Diagnostic Laboratory. A focally extensive area of the dorsal and ventral horn gray matter of the T8-T13 spinal cord segments was dark red (congestion and hemorrhage) (Fig. 1a). No lesions were apparent in the heart or other major parenchymal organs. An unfixed section of the pons and cerebellum sent to the Texas State Department of Health was negative for rabies via immunofluorescence assay (IFA). Tissues from all major organ systems were preserved in 10% neutral buffered formalin and routinely processed for paraffin embedding. Slides (5 μm sections) were stained with hematoxylin and eosin (HE). Histologically, small perivascular clusters of lymphocytes, fewer plasma cells and macrophages were scattered randomly throughout all levels of the spinal cord meninges, gray and white matter. A few axons near inflamed areas were mildly swollen with dilated myelin sheaths (spheroids). Lesions were most severe in the thoracic spinal cord within the reddened area observed at necropsy. Inflammatory cells occasionally surrounded pseudocysts in the white matter that contained a few 2–3 μm, round protozoan amastigotes (Fig. 1b) characterized by an outer thin periplasmic membrane, 1 μm basophilic nucleus and a rod-shaped kinetoplast (Fig. 1b inset) that was sometimes oriented parallel to nucleus. Amastigotes were not observed in the brainstem, cerebrum or cerebellum, and no histologic lesions were apparent in the heart and other parenchymal organs. The protozoa were not stained by periodic acid Schiff or Gomori methenamine silver stains. Unstained sections of affected areas of the spinal cord were sent to additional diagnostic laboratories for further identification of the protozoa. Although clusters of organisms were apparent in sections, the protozoa did not stain on immunohistochemistry for *Sarcocystis* spp. (University of Georgia Veterinary Diagnostic Laboratory; Fig. 1c), *Neospora* spp. or *Toxoplasma gondii* (United States Department of Agriculture, Beltsville). DNA *in situ* hybridization for *Leishmania* spp. (Michigan State University Diagnostic Center for Population and Animal Health) was also negative. Electron microscopy of the affected spinal cord was attempted, but the paucity of the protozoa and fixation artifacts prevented a thorough examination.

Sections (containing approximately 50 μm of tissue) from affected spinal cord and heart formalin-fixed paraffin embedded (FFPE) tissue blocks were processed for DNA extraction using two different methods: the E.Z.N.A® Tissue Extraction (Omega Bio-Tek, USA) and BiOstic® FFPE Tissue DNA Isolation (MO-BIO, USA) kits as per the manufacturers' instructions. DNA from the extracted horse samples was quantified using an Epoch spectrophotometer (Biotek, USA) with readings of 34–128 ng/μL DNA for the spinal cord samples and 800 ng/μL for the heart sample. Conventional polymerase chain reaction (PCR) was conducted using the TCZ1/TCZ2 (Moser et al., 1989) and Tc121/Tc122 (Virreira et al., 2003; Wincker et al., 1994) primer sets, which target the nuclear satellite and kinetoplast minicircle DNA sequences, respectively (Fig. 2). Quantitative real-time PCR (qPCR) was performed with the Cruzi 1/2 primer set with 6-carboxyfluorescein (FAM)-labeled Cruzi 3 probe (Duffy et al., 2013; Piron et al., 2007) as previously described except with an initial denaturation time of 3 min. FFPE heart tissue from a canine clinical isolate confirmed via histopathology and *T. cruzi* Sylvio X10 strain (ATCC©, USA) were used as positive controls, while negative controls consisted of distilled water and reaction mix with no template added. Positive bands were seen for the horse spinal cord samples with both conventional PCR primer sets. For qPCR, two separate extractions from a thoracic spinal cord block had positive cycle threshold (Ct) values of 27 and 24. Additional spinal cord sections from the thoracolumbar region had borderline positive Ct values (32 and 33). The heart sample was negative using all conventional and qPCR primer sets evaluated. The TCZ1/2 DNA fragment from the horse and the FFPE canine clinical isolate control were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and were sequenced offsite (Eton Biosciences, USA). The sequences were analyzed with the nucleotide Basic Local Alignment Search Tool (NCBI, USA) to determine homology. Both sequences had 98% identity with multiple *T. cruzi* accessions (AY520047.1, HM015662.1 and HM015648.1) in GenBank and corresponded to the *T. cruzi* satellite sequence as previously described (Moser et al., 1989). Further attempts to determine the *T. cruzi* discrete typing unit of the positive samples through amplification and DNA sequencing of the TcSC5D gene (Cosentino and Agüero, 2012) were unsuccessful, but the low sensitivity of this assay in samples