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Original article

Fatal *Halicephalobus gingivalis* infection in horses from Central America

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ABSTRACT

Halicephalobus gingivalis is a free-living nematode that causes an opportunistic infection in animals and humans. Two fatal cases of encephalitis and nephritis caused by *H. gingivalis* in equines from Costa Rica and Honduras are reported. Case 1: a 6-year-old Arabian stallion, from Costa Rica, presented severe neurological signs and was treated with systemic anti-inflammatory drugs and antibiotics. Because there was no improvement, it was euthanatized. Grossly, both kidneys showed large white nodules, ranging from 0.10 to 2.50 cm. Histopathologically, both kidneys showed similar changes consisting of multiple necrotic foci with longitudinal and transversal sections of nematode larvae. In the brain, there were several foci with similar parasites, surrounded by lymphocytes and gitter cells. Case 2: an 8-year-old Spanish stallion from Honduras it was reported as depressed and would not eat or drink water. The animal was treated with antibiotics and analgesics, without response and died spontaneously three days after the onset of clinical signs. Only pieces of kidney were sent for histopathological examination and showed findings similar to those described in case 1. These findings are similar with cases already reported expanding the knowledge about the geographical distribution of *H. gingivalis* in horses.

1. Introduction

Halicephalobus gingivalis (formerly *Micronema deletrix* and *Halicephalobus deletrix*) is a free-living and opportunistic nematode belonging to the order Rhabditida and family Panagroilamidae, commonly found in organic matter, such as soil and manure (Isazada et al., 2000; Akagami et al., 2007; Henneke et al., 2014). This organism causes a fatal infection primarily in horses, but sporadically also in zebras, bovines and humans (Isazada et al., 2000; Hermosilla et al., 2011; Enemark et al., 2016). Despite the severity of the disease, difficulty of *ante mortem* diagnosis and zoonotic potential (Lim et al., 2015; Enemark et al., 2016; Taulescu et al., 2016), knowledge regarding the life cycle, infection pathways and the pathogenesis of this nematode are scarce and limited (Taulescu et al., 2016).

The route of infection is believed to be through the oral mucosa, skin lesions or pulmonary infection via the inhalation of the nematodes (Ondrejka et al., 2010; Hermosilla et al., 2011; Adeyemi et al., 2015; Enemark et al., 2016). In horses, *H. gingivalis* affect different organs (brain, meninges, spinal cord, kidneys, eyes, mandible and maxillary bones, heart, blood vessels, lungs, testicles and preputium) where it causes granulomatous inflammations (Isazada et al., 2000; Muller et al., 2007; Henneke et al., 2014). Several authors have hypothesized that the dissemination occurs by haematogenous and lymphogenic routes and tissue migration (Henneke et al., 2014). Transmission from dam to foal, via colostrum, has also been reported (Wilkins et al., 2001). Only larval or female nematodes have been isolated from parasitized hosts (Hermosilla et al., 2011; Enemark et al., 2016) and the increase of the nematode number occur by parthenogenetic reproduction (Fonderie et al., 2013).

Several cases of halicephalobiasis in animals and humans have been reported in Australia (Lim et al., 2015), Asia (Akagami et al., 2007; Jung et al., 2014), Europe (Bryant et al., 2006; Henneke et al., 2014; Enemark et al., 2016; Taulescu et al., 2016), North (Rames et al., 1995; Isazada et al., 2000; Ferguson et al., 2008; Ondrejka et al., 2010) and South America (Sant'Ana et al., 2012). In this paper, are presented two additional cases from the Atlantic region of Central America (Costa Rica and Honduras), where no cases have been reported.

2. Materials and methods

2.1. Case 1

A six year-old Arabian stallion, from Costa Rica, was disoriented, circling left in its pen, and apparently blind, without indication of trauma. When the animal was walked in a straight line, the incoordination was more evident in the rear than in the fore limbs. It put its head against the pen wall. The right side of the upper and lower lips was dropping. The

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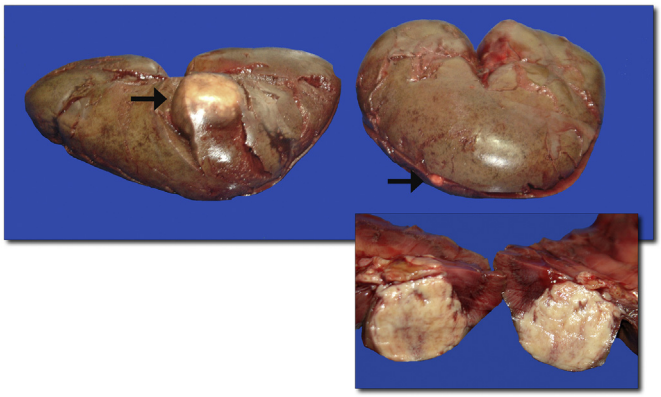

Fig. 1. Large and small white kidneys nodules (black arrow) involving mainly the cortical part. A sagittal section of the large nodule (inset).

tongue tonicity and the cervical sensitivity were diminished. No other nervous signs like trismus or prolapse of the third eyelid were observed. The CBC showed moderate leukocytosis, with 81% neutrophils and 19% lymphocytes. The blood smear was negative for hemoparasites. Serological test for *Leptospira* spp. was also negative. The animal was treated with systemic anti-inflammatory drugs and antibiotics. Because there was no improvement, it was euthanized at the farm after five days of clinical signs. The head, both kidneys and a piece of liver were sent for pathological examination. The brain, kidney and liver samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5-μm, and stained with hematoxylin and eosin. In addition, six scraping samples from the kidney were collected and stained with Giemsa. Three of them already stained were sent for parasitological identification.

2.2. Case 2

An eight year-old Spanish stallion from Honduras showed signs of depression and refused to ingest food or water. The animal was treated with antibiotics and analgesics, but there was no response and it died spontaneously three days after the onset of clinical signs. A necropsy was performed on the field sending only two small (0.50 and 1.50 cm) pieces of kidney for histopathological examination, which were processed, similar to case 1.

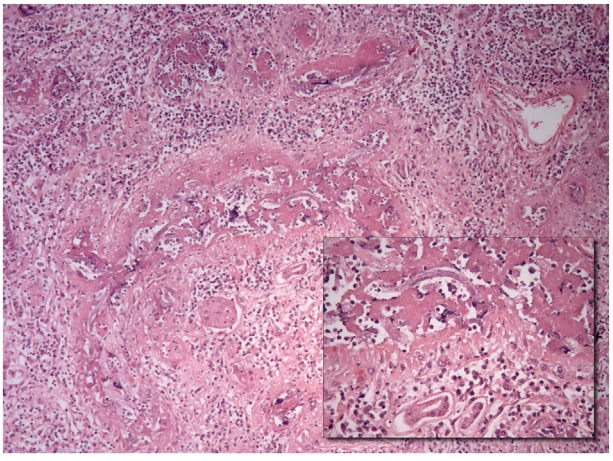

Fig. 2. Kidney. There is a large coagulative necrotic area with several parasitic segments. The inset two parasite section surrounding by the necrotic tissue. Hematoxylin and eosin.

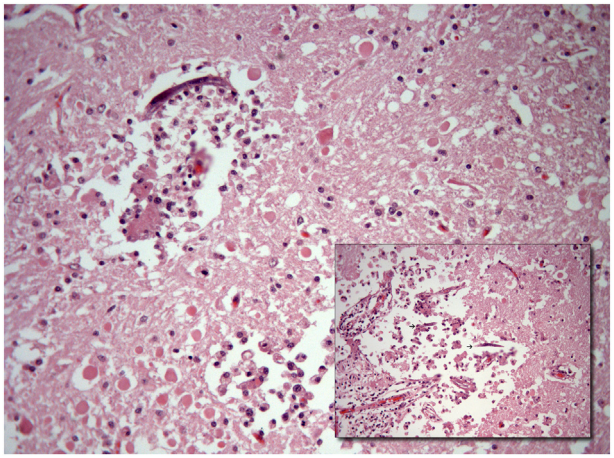

Fig. 3. Brain. In the center there is a longitudinal parasite segment surrounding by gitter cells. Furthermore, multiple necrotic eosinophilic bodies are seen. The inset with two parasites sections (black arrows). Hematoxylin and eosin.

3. Results

3.1. Case 1

Grossly, the brain and liver did not have alterations. Both kidneys showed mainly in the cortical area large white nodules, ranging from 0.10 to 2.50 cm diameter (Fig. 1).

Histologically, both kidneys showed similar changes consisting of multiple necrotic foci with longitudinal and transversal sections of nematode larvae (Fig. 2). In the brain, there were several foci with similar parasites, surrounded by lymphocytes and gitter cells (Fig. 3).

Cytologically, several tangential nematodes, mixed with mononuclear cells, epithelial cells and fibrocites were seen (Fig.4).

3.2. Case 2

Macroscopically, the two kidney samples showed diffuse white areas with little kidney tissue in their edges. The microscopic findings were similar to those described in case 1.

A large numbers of nematodes of different stages (larvae and adult) were found. Females were 300–350 μm in length and 15–20 μm in diameter and had a cylindrical body with tapered head anterior end and tail, and a rhabditiform esophagus with the characteristic corpus, isthmus, and valved bulb. The larval stages were smaller (125–250 μm) and had the same features as the fully developed worms but lacked a

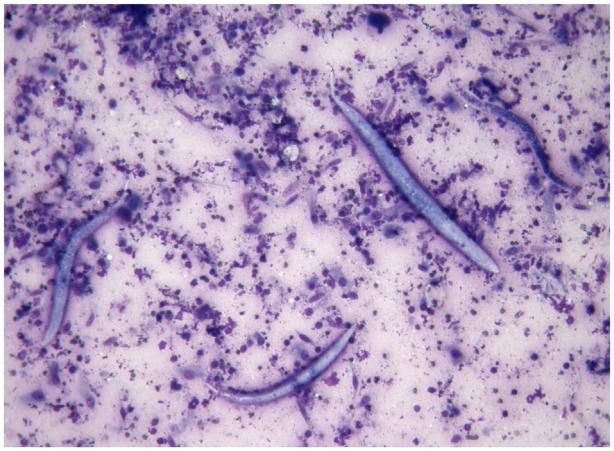

Fig. 4. Four parasite surrounding by inflammatory cells, cellular detritus and fibrocites.

reproductive system. These morphologic features are consistent with the description of *H. gingivalis*.

4. Discussion

The clinical signs associated with *H. gingivalis* infection are very variable, depending on the localization of the parasites. Among the most affected organs are the CNS (meningoencephalomyelitis) (Bryant et al., 2006; Akagami et al., 2007; Hermosilla et al., 2011; Sant'Ana et al., 2012; Jung et al., 2014; Adeyemi et al., 2015; Taulescu et al., 2016) and the kidneys (nephritis) (Akagami et al., 2007; Taulescu et al., 2016) as was the case of the horses included in the present study. In addition, for the first time an outbreak of bovine meningoencephalomyelitis has been reported (Enemark et al., 2016). In humans, *H. gingivalis* cause fatal meningoencephalitis (Ondrejka et al., 2010; Lim et al., 2015). The route by which the animals acquired the *H. gingivalis* infection is unknown. Interestingly, both horses were from the Atlantic region, a very humid region. Also both cases occurred during the rainy season, when climate conditions may be favorable for the life cycle of the parasite in the soil or manure. *Ante mortem* diagnosis is a major challenge of the halicephalobiasis due to the lack of sensitive diagnostic and conclusive clinical parameters. In humans the majority of cases with systemic clinical signs (especially encephalitis) are diagnosed post mortem with only one case diagnosed *ante mortem* (Adeyemi et al., 2015). Although uncommon, the infection by *H. gingivalis* should be considered in the differential diagnosis of horses with neurological signs. The presented cases confirm the occurrence of this nematode for the first time in Central America and also raise public health awareness, as the parasite is zoonotic.

Declaration of conflicting interests

The authors declare no conflicts of interest.

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