Arch Med Vet **46, 407-411 (2014)**

ORIGINAL ARTICLE

Maxillary osteomyelitis due to *Halicephalobus gingivalis* and fatal dissemination in a horse

Osteomielitis maxilar debido a *Halicephalobus gingivalis* y diseminación fatal en un caballo

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RESUMEN

En la presente comunicación se expone un caso de infestación parasitaria poco habitual causada por *Halicephalobus gingivalis*, cuya manifestación principal fue osteomielitis del hueso maxilar. El caballo mostraba inicialmente inflamación y dolor en la región de la cresta facial derecha. Las radiografías demostraron la presencia de osteolisis y ensanchamiento de la cresta facial. La biopsia del hueso mostraba inflamación granulomatosa y un gran número de larvas del nematodo. El caballo fue tratado con ivermectina. Inicialmente mejoraron los signos clínicos, pero dos meses y medio después el caballo desarrolló uveítis y fallo renal, por lo que fue eutanasiado. El estudio anatomopatológico mostró múltiples granulomas parasitarios en los riñones y en la úvea. La infección por *Halicephalobus gingivalis* es poco frecuente en caballos y personas aunque presenta una distribución mundial. De acuerdo con los autores esta es la primera vez que se describe dicha infestación en un équido en España.

Palabras clave: *Halicephalobus gingivalis,* caballo.

SUMMARY

This study reports a rare case of maxillary osteomyelitis in a horse caused by *Halicephalobus gingivalis*. The horse presented inflammation and pain in the region of the right facial crest and the radiographs detected osteolysis and widening of the facial crest. The biopsy revealed a granulomatous inflammation and a large amount of parasite larvae. The horse was treated with ivermectin but it developed uveitis and renal insufficiency 2.5 months later and was euthanised. The anatomopathological study found multiple parasitic granulomas in the kidneys and uveal tract. *H. gingivalis* is an infrequent infection in horses and people, and it has a worldwide distribution. To the best of our knowledge this is the first report of *H. gingivalis* infection in an equid to be diagnosed in Spain.

Key words: *Halicephalobus gingivalis*, horse.

INTRODUCTION

Halicephalobus gingivalis is a panagrolaimid nematode from the order Tylenchida (formerly Rhabditida). They are free-living organisms with a wide geographic distribution (Anderson *et al* 1998) which are capable of facultative parasitism and can cause opportunistic infections in horses (Blunden *et al* 1987). The infection and life cycle are scarcely understood. Previous reports describe infection through wounds (Gardiner *et al* 1981, Eydal *et al* 2012), inhalation or ingestion (Blunden *et al* 1987), trans-mammary from dam to foal (Wilkins *et al* 2001) and posterior dissemination through the blood stream (Yoshihara *et al* 1985, Kinde *et al* 2000). After the nematodes enter a horse, they reproduce via parthenogenesis which causes granulomatous inflammation and destruction of host tissues (Ruggles *et al* 1983). They can cause meningoencephalitis (Bryant *et al* 2006), nephritis, gingivitis, osteomyelitis, posthitis, orchitis, papillitis, retinitis (Hermosilla *et al* 2011), uveitis, (Kinde *et al* 2000), ocular parasitism (Rames *et al* 1995), radiculomeningomyelitis (Johnson *et al* 2005) and arthritis (Simpson *et al* 1988). The condition is usually fatal and there are only three reports of successful treatment following the administration of ivermectin an antiparasitic drug, although the lesions did not affect the central nervous system (CNS) (Dunn *et al* 1993, Simon *et al* 2001, Schmitz and Chaffin 2004, Ferguson *et al* 2008).

MATERIAL AND METHODS

An 11-year-old, crossbred gelding was referred to our institution with signs of enlargement of the retropharyngeal lymph nodes for 3 months, deformation of the facial crest, and sero-sanguinolent discharge from the right nostril during the previous 5 days. The suspected diagnosis was sinusitis.

On arrival, the physical examination detected inflammation, pain, and a small scar in the region of the right facial crest, enlargement of the right retropharyngeal lymph nodes and sero-sanguinolent discharge from the right nostril.

Radiographs were taken and the dorso-ventral view included an enlarged and osteolytic facial crest with a

Accepted: 19.12.2013.

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Table 1. Hematological and biochemical parameters.
Valores hematológicos y bioquímicos.

	Day 1	Day 75	Reference range
RBC x10^6 /µL	6.61	9.26	6.5-12.5
PCV %	27.7	37	32-48
WBC x10^3 /µL	7	8.8	6-12
Neutrophils %	62	51	30-65
Lymphocytes %	35	38	25-70
Eosinophils %	3	11	0-11
Platelets x10^3 /µL	201	1161	100-600
Total Proteins g/dL	7.8	9.8	4.6-6.9
BUN mg/dL		66	12-45
Creatinine mg/dL		4	0.5-1.7
GGT UI/L		15	12-45
Billirubine mg/dL		1.3	0.1-1.9

honeycomb-like appearance (figure 1). Fluid lines were not observed in the latero-lateral view of the maxillary or frontal sinuses.

Ultrasonographic examination of the facial crest detected discontinuation of the bone in an area of at least 10 cm, which was compatible with microfractures and osteolysis.

Endoscopic examination of the nasal passages did not detect discharge from the naso-maxillary opening.

The hematological and biochemical parameters were within the normal range (table 1).

A bone biopsy was conducted under local anesthesia. One sample was cultured for microbiologic identification. *Candida* spp. was isolated but it did not correlate with the histopathological findings. Histopathological analysis was performed using paraffin sections, which were stained routinely with hematoxylin and eosin. The light microscopic examination detected high-cellularity tissue and a granulomatous inflammation with giant multinucleated cells, lymphocytes, plasmatic cells, eosinophils and large amounts of parasite larvae (figure 2). In other sections, the bone tissue had normal characteristics. The cytological study of the right retropharyngeal lymph-node after fine needle aspiration found necrosis, cell degeneration, and a few lymphocytes.

The distinctive morphology of these larvae allowed the identification of *H. gingivalis* nematodes. The treatment comprised ivermectin, ketoconazol (for 2 months) and phenylbutazone. The clinical signs of pain and inflammation improved almost completely.

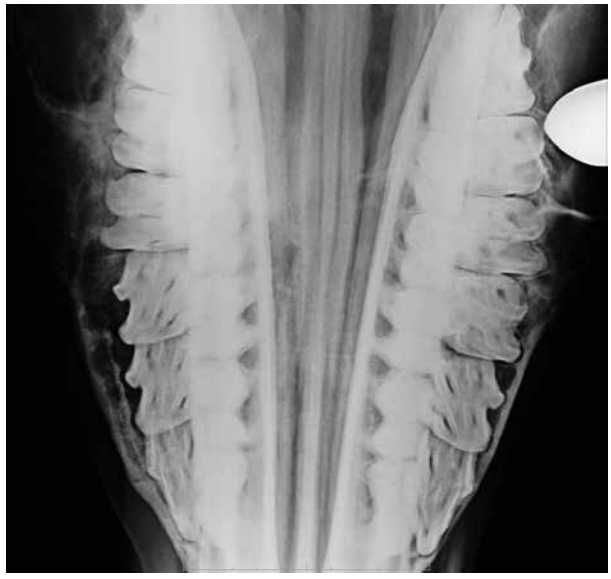

Figure 1. Dorso-ventral view of the skull showing the osteolytic facial crest with a honey-comb-like appearance**.** X-ray parameters: 80 mAs, 90 kV.

Proyección dorsoventral de la cabeza donde se aprecia la cresta facial con osteolisis y apariencia de panal de miel. Exposición radiológica: 80 mAs, 90 kV.

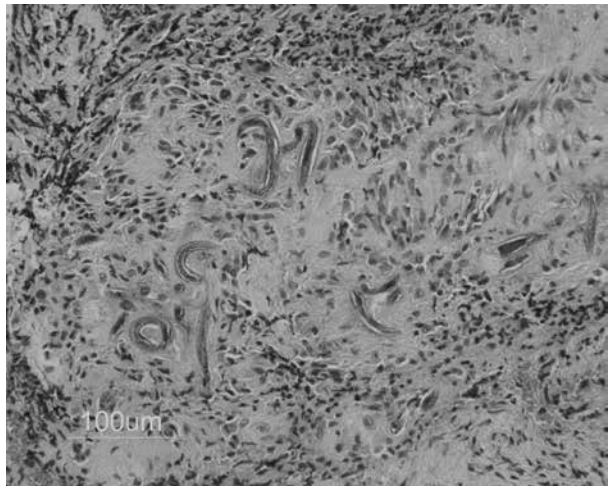

Figure 2. Granulomatous inflammation with lymphocytes, plasma cells, macrophages, fibroblasts and a large amount of *Halicephalobus gingivalis* larvae. Biopsy of the facial crest. H-E (×20).

Inflamación granulomatosa con linfocitos, células plasmáticas, macrófagaos, fibroblastos y una gran cantidad de larvas de *Halicephalobus gingivalis*. Biopsia de la cresta facial. H-E (×20).

RESULTS AND DISCUSSION

The horse returned to our facilities 2.5 months later for follow-up control and the radiological and ultrasonography findings were similar to those during the first visit.

During the control visit, the horse had right unilateral uveitis. The surface of the left kidney was irregular on rectal palpation. The horse also presented pigmenturia and the urine analysis detected hematuria (200 cells/µL), proteinuria (0.3 g/L), 70 leukocytes per µL, and a urinary density of 1008 mg/mL. The hematology results indicated an elevated total protein level (9.8 g/dl), eosinophilia (11%) and an elevated platelet count (1,161x10^3 /µl) (table 1). The biochemistry findings indicated chronic renal failure; thus the horse was euthanized because of the poor prognosis.

A subsequent anatomopathological study found multiple nodules measuring 1-6 cm in diameter in both kidneys, which were pale, rounded and solid in appearance (figure 3), and distributed in the cortical and medular areas. The histopathological analysis of the kidney demonstrated a loss of the renal parenchyma architecture and a granulomatous inflammation with lymphocytes, plasma cells, macrophages, eosinophils, and *H. gingivalis* larvae (figure 4). Several larvae and a lower level inflammatory reaction were found in the uveal tract (figure 5). Some nematodes were also observed within the vascular lumens.

All of the parasites found in the different anatomical locations shared the same morphological characteristics but were at different stages of the life cycle. They were mainly larvae and only female nematodes were seen among the adult forms. The size of the larvae varied between 75 and 250 µm in length and 7.5 µm in width. The adult female width reached 15-19 µm. The length/width ratio ranged from 11/1 in the larvae to 15/1 in the adult nematodes.

The adult forms had a smooth cuticle, a cylindrical body, and a slightly conical anterior end, which had an extended cylindrical mouth with dimensions of 6-2 µm. The characteristic rhabditiform esophagus occupied the first third of the body. The esophagus had a corpus, an isthmus, and a valve bulb with a relative length ratio of 16/12/6. These morphological features confirmed the diagnosis of *H. gingivalis* infection (figure 6).

H. gingivalis is a rare infection in horses and people. This facultative parasite has a worldwide distribution but

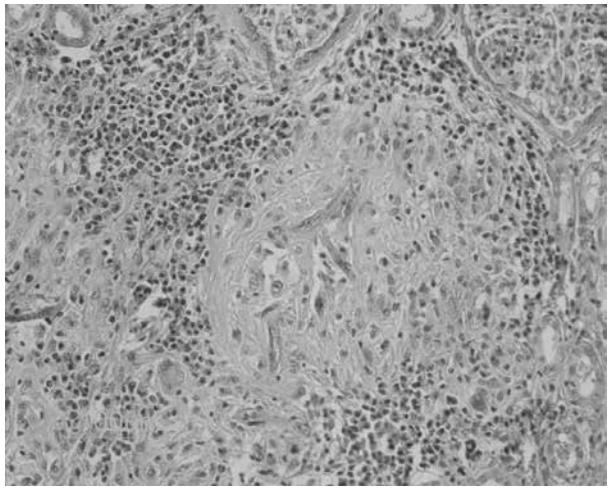

Figure 3. Granulomatous inflammation with lymphocytes, plasma cells, macrophages, eosinophils, and *Halicephalobus gingivalis* larvae in the kidney. H-E (×20).

Inflamación granulomatosa con linfocitos, células plasmáticas, macrófagos, eosinófilos y larvas de *Halicephalobus gingivalis* en el riñón. H-E (×20).

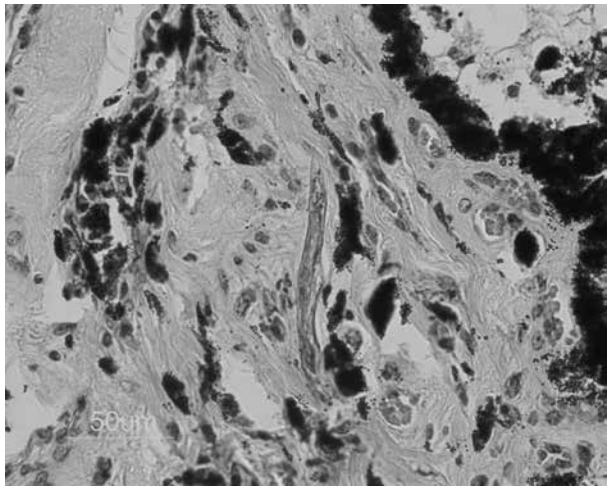

Figure 4. Parasitic *Halicephalobus gingivalis* larvae in the uveal tract. H-E (×40).

Larvas parasitarias de *Halicephalobus gingivalis* en la úvea. H-E (×40).

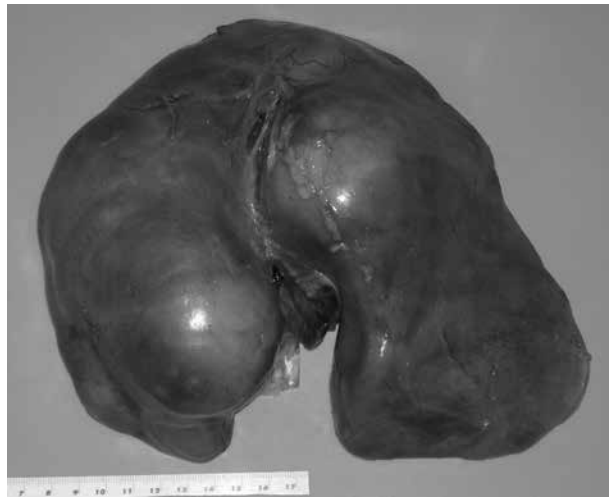

Figure 5. Pale, rounded and solid parasitic granulomas in the kidney.

Granulomas parasitarios pálidos, redondeados y sólidos en el riñón.

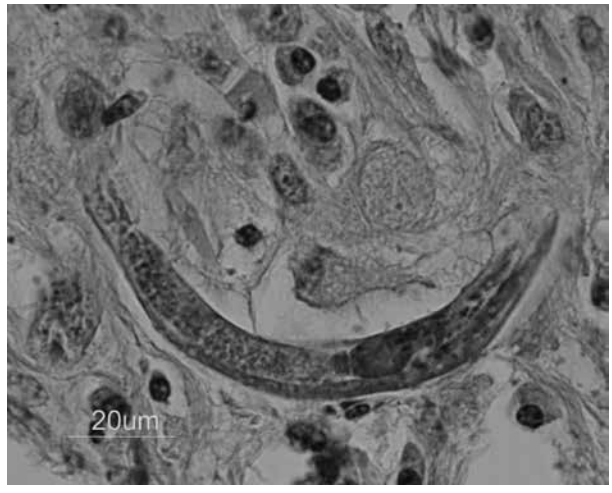

Figure 6. Image of *Halicephalobus gingivalis* showing the characteristic anterior end.

H-E (×100). *Halicephalobus gingivalis* mostrando su característica terminación anterior. H-E (×100).

this case is the first to be diagnosed in Spain. The mode of infection, pathogenesis, distribution, and prevalence of this parasite are all enigmatic and they require further study. In this case, we suspect that the infection may have entered via a wound over the facial crest. The wound had already healed but the clinical signs appeared much later, which was similar to the report by Eydal *et al* (2012).

As mentioned earlier, the organs that are affected most frequently in the horse include the brain, kidneys, oral and nasal cavities (Blunden *et al* 1987), lymph nodes, spinal cord and adrenal glands. Other tissues affected may include the heart, stomach, liver, ganglia and bone (mandible, maxilla, femur, and nasal bones) (Spalding *et al* 1990). In the current case, we confirmed lesions in the maxilla, kidneys, uveal tract, and lymph nodes, which were sampled for microscopic evaluation. The only exception was the central nervous system. Despite the lethargic behaviour of the horse we did not observe any macroscopic lesions so further examinations were not performed. It would have been beneficial to make a more detailed examination of the brain but due to the lack of other neurological signs, we assumed that the general condition of the horse and renal failure were responsible for the lethargy. Lethargy has been described as one of the symptoms in the four previously reported medical cases, altough other remarkable CNS-related symptoms were also present, such as neck pain, headache, confusion, and convulsions (Ondrejka *et al 2010*).It should also be noted that in people and horses, the clinical signs progress very rapidly when the brain is affected by the parasite. These symptoms/signs are related to the tropism for the CNS. After reaching the brain, damage is produced by the inflammatory reaction of the host which usually involves the infiltration of mononuclear cells (including macrophages, plasma cells and neutrophils) and multinucleated inflammatory cells. These inflammatory cells can also form micro-abscesses and granulomas (Pearce *et al* 2001, Johnson *et al* 2001, Ondrejka *et al* 2010, Eydal *et al* 2012). Other organs lacked macroscopic lesions or clinical findings that suggested lesions on them.

The dissemination route within the host is unknown in the present case. However, we consider that the parasite spread via the blood-stream because some nematodes were observed within the blood vessels of the kidney (Yoshihara *et al* 1985, Kinde *et al* 2000).

There is agreement regarding the poor prognosis for this condition. It has been hypothesized that the inability of antihelmintics to cross the blood-brain barrier (Plumb 2002), the lack of sensitivity to ivermectin therapy in *H. gingivalis*, the difficulty of obtaining an appropriate *antemortem* diagnosis in the absence of visible granulomatous lesions and the rapid evolution of the condition - especially when the nervous system is involved - may contribute to the poor prognosis (Ferguson *et al* 2008, Hermosilla *et al* 2011). In this particular case, we speculate that the prognosis for recovery was poor due because the nematode was protected from high concentrations of the drug by bone sequestration (Bröjer *et al* 2000), as well as the severe and rapid progression of the insult to the kidneys.

We conclude that *H. gingivalis* infection should be included in the differential diagnosis of osteolytic lesions of the head. Cutaneous lesions may be the site of entry and a guarded to bad prognosis is anticipated.

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