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Equine meningo-encephalitis caused by *Halicephalobus gingivalis*: a case report observed during West Nile disease surveillance activities

Gabriella Di Francesco[1], Giovanni Savini[1], Alessandro Maggi[2], Nicola Cavaliere[3], Anna Rita D'Angelo[1] & Giuseppe Marruchella[1]

Summary

A seven-year-old horse was euthanised after exhibiting a severe and rapidly progressive neurological disorder. Tissue samples were despatched to the Italian Reference Centre for Animal Foreign Diseases (*Istituto 'G. Caporale'* in Teramo) for diagnosis. All laboratory tests for equine neurotropic viruses gave negative results. Scattered perivascular inflammatory infiltrates and several parasites that were morphologically classified as *Halicephalobus gingivalis*, were seen within the brain upon microscopic examination. Pathological findings led to the diagnosis of parasitic meningo-encephalitis caused by *H. gingivalis*. This case report confirms that halicephalobosis should be taken into account in the differential diagnosis of equine encephalopathy and it also highlights the value of a multidisciplinary approach to problem solving in veterinary medicine.

Keywords

Halicephalobus gingivalis, Horse, Italy, Meningo-encephalitis, Parasite, Surveillance, West Nile disease.

Introduction

Equine encephalopathies are relatively uncommon and can be caused by different aetiological agents, namely: viruses (e.g. *Flaviviruses*, *Herpesviruses*, *Alphavirus*), bacteria (e.g. *Streptococcus equi*), protozoa (e.g. *Sarcocystis neurona*, *Neospora* spp.), parasites (e.g. *Halicephalobus gingivalis*, *Setaria* spp., *Strongylus vulgaris*), toxic substances (e.g. fumonisins) and metabolic disorders (e.g. hypocalcemia and hepatic encephalopathy) (20).

Recently, special attention has been focused on West Nile disease given its zoonotic potential and due to its extensive geographic distribution. In Italy, West Nile disease was firstly described in Tuscany in 1998 (6) and re-emerged in 2008 in the Po Basin (17). West Nile disease outbreaks occurred annually in Italy between 2008 and 2011, affecting new areas and host populations (11).

Italian legislation on West Nile disease (2) requires that equids that exhibit nervous symptoms and/or those that have died after a neurological incident must be subjected to specific laboratory tests to confirm or, alternatively, to rule out the presence of West Nile disease. This paper describes the diagnostic approach and results obtained in a horse recently suspected of being affected by West Nile disease in Italy.

Materials and methods

In February 2012, a seven-year-old male horse (Arabian × quarter horse) from near Foggia (southern Italy) showed a severe, rapidly

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', via Campo Boario, 64100 Teramo, Italy
g.difrancesco@izs.it
(2) Veterinary Practitioner, via Piave 78, 71121 Foggia, Italy
(3) Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, via Manfredonia 20, 71121 Foggia, Italy

progressive neurological disorder. Traumatic secondary skin lesions were also observed. The horse that was permanently recumbent was euthanised. As specified by the above-mentioned Italian legislation on West Nile disease (2), tissue samples, either refrigerated (blood, brain, cervical and lumbar spinal cord, heart and spleen) or fixed in 10% neutral buffered formalin (brain and spinal cord), were collected and despatched to the Italian Reference Centre for Animal Foreign Diseases (*Istituto 'G. Caporale'* in Teramo) for diagnostic investigation.

Virological and serological investigations

Samples collected *intra vitam* (blood, serum, plasma) or post mortem (brain, spinal cord, heart, spleen) were used for virus isolation in susceptible cell cultures (RK13, Vero, C6/36). Specific biomolecular investigations were also performed to detect the genome of West Nile disease virus (24) and equine herpesvirus types 1 and 4 (3).

Serological tests were conducted to identify a specific anti-West Nile disease virus immune response. A commercially available enzyme-linked immunosorbent assay (ELISA) kit was used for IgM antibodies (IgM West Nile disease, IDEXX, Montpellier), whereas IgG were tested by ELISA (22). Seroneutralisation and plaque reduction neutralisation assays were performed to confirm previous results (11, 13, 14).

Histopathology

Tissue samples from different sections of the central nervous system (CNS) were collected and embedded in paraffin. Tissues were sectioned in 5-µm slices and subsequently stained with haematoxylin and eosin. Retrospectively, aliquots of the formalin solution where the CNS had been fixed were centrifuged (300 × g for 5 min). The sediment was then eluted with phosphate buffer solution, placed on glass slides, stained with Lugol's iodine solution and examined by light microscopy.

Results

Virological and serological investigations

Virus isolation by cell culture and biomolecular studies demonstrated no viral infection. Serological investigations demonstrated the presence of specific anti-West Nile disease virus IgG (1:80), whereas IgM were not detected. Since IgM are no longer detectable three months post infection, serological data indicated a previous, but not recent, infection.

Histopathology

Microscopically, a number of perivascular cuffs were observed, mainly involving the pons and the midbrain. Inflammatory infiltrates consisted of lymphocytes and macrophages, which were sometimes organised in microgranulomas, whilst eosinophils were observed occasionally (Fig. 1). Several parasites were detected in the blood vessels, in the perivascular spaces or, more rarely, free in the brain parenchyma. Morphological features of such parasites (size and shape) were similar to those reported in the literature for *H. gingivalis* (Figs 2 and 3). Furthermore, scattered ring-shaped haemorrhages that were observed macroscopically during brain sampling (Fig. 4), along with the presence of parasites and inflammation in the meninges (Fig. 5) were also recorded.

Nematodes that were morphologically consistent with *H. gingivalis* were also detected upon microscopic examination of the fixative sediment. Furthermore, 0-3 parasitic bodies were observed in 100 µl of sediment; their length was approximately 200-400 × 15-20 µm. The rhabditiform oesophagus of *H. gingivalis* could be identified with ease (Figs 6a and 6b). The pathological findings described resulted in the diagnosis of meningo-encephalitis caused by *H. gingivalis*.

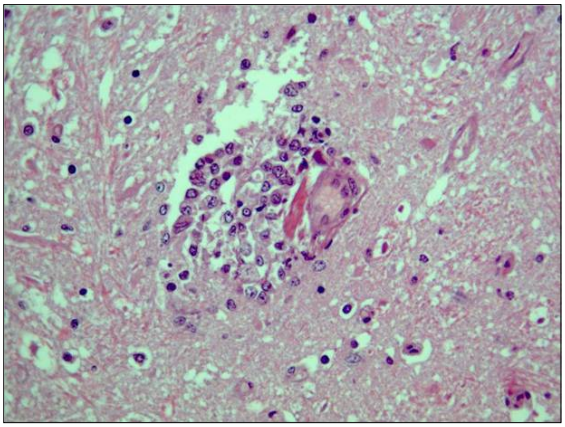


Figure 1
Horse midbrain: perivascular cuffing in microgranulomas
Haematoxylin and eosin (×40)

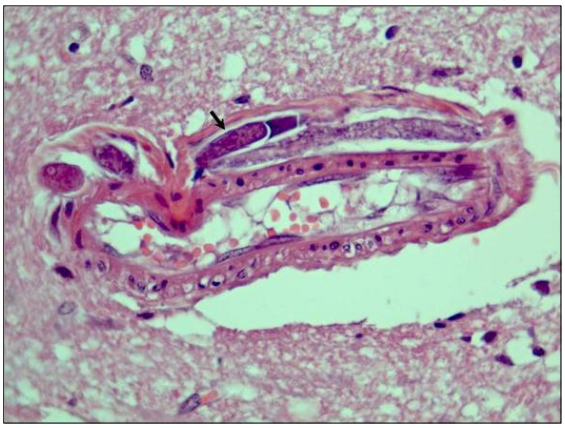


Figure 2
Horse midbrain: parasitic bodies morphologically identified as *Halicephalobus gingivalis* situated close to a blood vessel
A mature female nematode contains an uninucleate egg with a prominent nucleolus (arrow)
Haematoxylin and eosin (×63)

Discussion

Infections caused by *H. gingivalis* occur infrequently in horses and humans and, very rarely, in other mammals (9, 10, 19). To the best of our knowledge, approximately 50 cases of halicephalobosis have been reported (8), including those observed in Italy (5, 7, 15, 16).

The *H. gingivalis* parasite (also known as *H. deletrix* and *Micronema deletrix*) is distributed worldwide and belongs to the order Rhabditida, which usually lives as a saprophyte in soil and in decaying organic humus. The life-cycle of *H. gingivalis* is poorly understood and probably consists of two

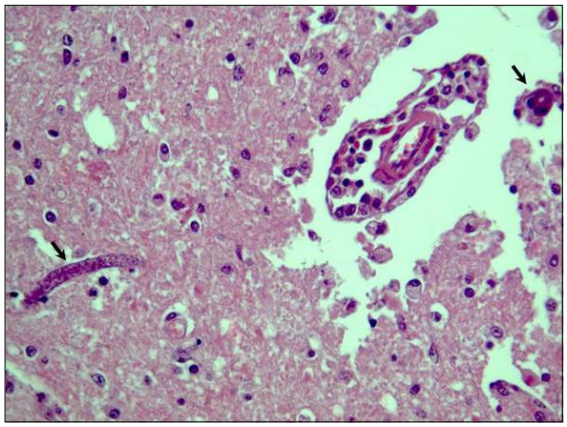


Figure 3
Horse pons: a blood vessel surrounded by prominent inflammation
Two parasites are also observed (arrows)
Haematoxylin and eosin (×40)

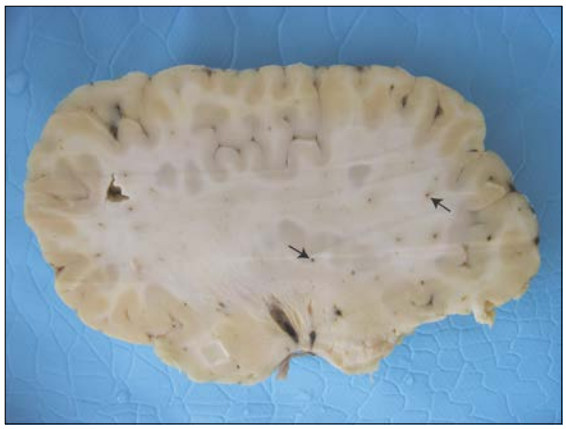


Figure 4
Horse brain showing scattered petechiae within the brain (arrows)

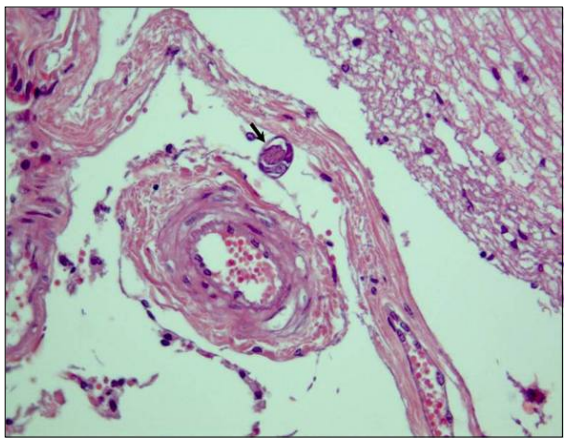
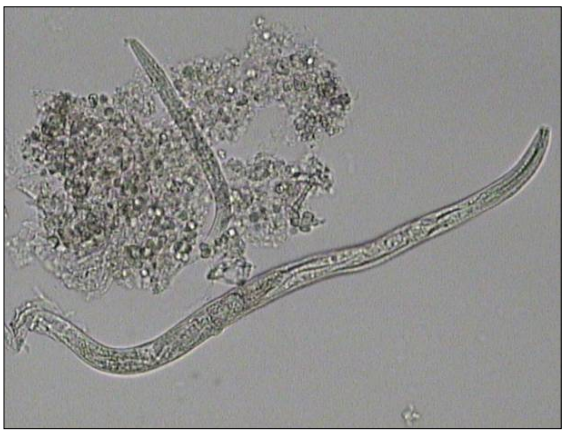


Figure 5
Horse midbrain with a cross-sectioned nematode located close to a meningeal blood vessel
Haematoxylin and eosin (×40)

(a) Two parasites are demonstrated (x40)



(b) Bulb of the rhabditiform oesophagus (arrow)

Figure 6
Formalin sediment in a horse
Lugol's iodine solution stain (×40)

distinct phases, namely: a parthenogenic phase in mammals and a sexual phase in the environment (18). The pathogenesis of halicephalobosis is also largely unknown. Infection probably occurs through the mucous membranes and/or the skin, although other paths of infection cannot be ruled out (9, 23). *H. gingivalis* then spreads via the haematogenous route, showing a specific tropism for the CNS (4, 9). In this case report, the skin lesions observed are more likely the result of neurological symptoms and not the portal of entry of parasites, whilst the presence of parasites within and/or close to the blood vessels further supports the pathogenetic hypothesis of the dissemination through the body via the bloodstream (4, 5, 9, 12).

Meningo-encephalitis caused by *H. gingivalis* involves rapidly progressive disorders and lesions can involve the entire CNS or, as in the present case, specific areas of the brain, thus influencing clinical signs. Microscopically, *H. gingivalis*-induced lesions mainly consist of perivascular cuffs created by macrophages, multinucleated giant cells, lymphocytes, plasma cells and eosinophils (4, 5, 9, 15).

Ante mortem diagnosis is possible when *H. gingivalis*-induced granulomas are accessible (skin and oral lesions) or with those that affect the kidneys. In the latter case, parasites can be detected in the urinary sediment (12). By contrast, neurological halicephalobosis can only be diagnosed post mortem by histopathology (3, 18). As reported previously (5, 12) and confirmed in the present case report, *H. gingivalis* can be detected with ease in the fixative sediment. Consequently, microscopic examination of the formalin sediment can be a useful, preliminary diagnostic test. It is worth noting that certain morphological features, such as the rhabditiform oesophagus and the shape of uterus, differentiate *H. gingivalis* from other neurotropic parasites (e.g. *Angiostrongylus cantonensis*, *S. vulgaris*, *Setaria* spp. and *Parelaphostrongylus tenuis*) (1, 5, 8, 12, 15).

Since diagnosis of neurological halicephalobosis is confirmed post mortem, any targeted therapy is unrealistic. Furthermore, anthelmintic drugs are unsuccessful since they do not effectively pass the blood-brain barrier and penetrate granulomas (21). To date, there are no specific guidelines for the prevention of equine halicephalobosis. However, the hygiene of the environment and of skin lesions may reduce the risk of infection.

Conclusions

This case report suggests that parasitic meningo-encephalitis should be considered in the differential diagnosis of equine encephalopathy and highlights the value of a multidisciplinary approach to problem solving in veterinary medicine, particularly where neurotropic and/or zoonotic viruses are present. The former circulation of West Nile disease virus near Foggia, as confirmed by our serological data, resulted in the presumptive diagnosis of West Nile disease. Surveillance

activities for West Nile disease could significantly contribute to the study of equine encephalopathies, including neurological disorders that are currently underestimated on account of their clinical and pathological features.

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