First Human Case of Fatal *Halicephalobus gingivalis* Meningoencephalitis in Australia

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*Halicephalobus gingivalis* (previously *Micronema deletrix*) is a free-living nematode known to cause opportunistic infections, mainly in horses. Human infections are very rare, but all cases described to date involved fatal meningoencephalitis. Here we report the first case of *H. gingivalis* infection in an Australian human patient, confirmed by nematode morphology and sequencing of ribosomal DNA. The implications of this case are discussed, particularly, the need to evaluate real-time PCR as a diagnostic tool.

**CASE REPORT**

A 74-year-old lady from a remote town in the Eyre Peninsula of South Australia with a 4-day history of mental state deterioration, fever, and a loss of coordination was transferred to the Royal Adelaide Hospital. She was moderately immune suppressed by methotrexate and etanercept treatment for rheumatoid arthritis and had a history of diabetes. During the admission, her conscious state deteriorated rapidly, requiring mechanical ventilation and admission to the intensive care unit (ICU). Subsequently, she developed signs of brainstem involvement and exhibited a loss of corneal and gag reflexes. She was administered benzylpenicillin, ceftriaxone, and aciclovir for presumptive meningoencephalitis of bacterial or viral etiology. Cerebrospinal fluid (CSF) obtained by lumbar puncture demonstrated $2.8 \times 10^6$ polymorphonuclear leukocytes (PMN)/liter, $1.8 \times 10^6$ mononuclear lymphocytes/liter, elevated CSF protein of 1.59 g/liter, and CSF glucose of 3.3 mmol/liter; aerobic and anaerobic bacterial culture results were negative, and no bacteria detected upon Gram staining. CSF stained with Diff-Quick (Aler, Brisbane, Australia) showed 99% PMN with very few eosinophils. CSF protein was markedly elevated (5.46 g/liter), and CSF glucose was 1.3 mmol/liter. Microscopic examination of 100 µl of unstained CSF was performed after centrifugation at 700 × g for 10 min, but no amoebic trophozoites were detected. With a suspicion of parasitic infection, given the unexplained high PMN counts, the antimicrobial treatment strategy was changed to liposomal amphotericin B, sulfadiazine, pentamidine, and azithromycin to target protists such as amoebae and *Toxoplasma gondii*. CSF was subjected to PCR for *Naegleria fowleri*, *Acanthamoeba* sp., and *Balamuthia mandrillaris*, but all test results were negative. No anti-*Strongyloides* serum antibody (IgG) was detected in an enzyme-linked immunosorbent assay using somatic larval antigens from *Strongyloides ratti* (Bordier Affinity Products) (1, 2). At day 7 of admission, the patient died following a complete loss of brainstem functions.

An etiological diagnosis was made based on postmortem findings. Microscopy of CSF and brain tissue exhibited numerous motile nematodes containing oval, elongated, thin-shelled, colorless eggs of 40 to 55 µm by 20 to 25 µm in size (average of 10 eggs) (Fig. 1). The larvae in CSF were 250 to 300 µm long and 15 to 20 µm wide, with a rhabditoid esophagus (70 to 90 µm long). Larvae from CSF were cultured using a modified *Strongyloides* agar plate culture method by replacing fecal material with an *Escherichia coli* ATCC 25922 suspension together with 100 µl of CSF onto the middle of a Mueller-Hinton agar plate (Oxoid, Australia) (3, 4). Every 7 days, new plates were inoculated. *E. coli* grew in tracks created by motile nematodes as they moved out of the central inoculum, and microscopic examination revealed nematodes at different stages of development (Fig. 2). Only female adult worms were observed; they possessed didelphic reproductive tracts and reflexed ovaries at the posterior end, consistent with the description of *Halicephalobus gingivalis* (5). The live nematodes were fixed in ethanol. Subsequently, DNA was isolated from individual worms and subjected to PCR-based sequencing of nuclear large-subunit ribosomal DNA (LSU rDNA) (6). The sequences deter-
mined from four individual nematodes were all the same and had 99% homology (1,385/1,399 bases) to that of *H. gingivalis* SAN100, isolated from a horse in Guelph, Canada (GenBank accession no. AY293177.1 [7]).

A complete postmortem examination was conducted, and a study of the brain revealed congested leptomeningal blood vessels without significant opacity of the leptomeninges or CSF. The brain had a normal weight of 1,160 g, and there was no significant cerebral edema. There was extensive brain necrosis, primarily affecting the temporal lobes (bilaterally) and the right and left basal ganglia, anterior corpus callosum, right cerebral peduncle, and cerebellum. Histopathological examination of the brain showed meningoencephalitis, with mild to moderate perivascular inflammation comprising lymphocytes and macrophages and with no evidence of granulomatous inflammation (Fig. 2). The inflammation extended into the brain parenchyma, and there were multiple foci of necrosis and widespread cortical hypoxic-ischemic injury characterized by neuronal red cell change. Adult female nematodes, larvae, and eggs were observed in every section of the brain (bilateral hemispheres, cerebellum, brain stem, pituitary gland, and leptomeninges), primarily in the perivascular spaces, including areas within the brain parenchyma in which the presence of *H. gingivalis* was identified without any apparent associated inflammatory response. The spinal cord was not examined. The nematode was not observed in any other organs (including heart, lungs, liver, and kidneys).

*H. gingivalis* belongs to the nematode family Paragrolaimidae. Currently, there are eight described species of *Halicephalobus*, and only *H. gingivalis* has been reported to infect humans and equines, predominantly horses (5) (Table 1 and Table 2). Only female worms have been isolated from parasitized hosts, confirming that *H. gingivalis* can reproduce parthenogenetically, although how *H. gingivalis* infects human and equine hosts is unknown (8–12). In

![FIG 1](A to C) Iodine stain of CSF obtained postmortem shows different stages of *H. gingivalis* egg development. (A) Single-cell stage (1 scale unit = 2.5 μm). (B) Two-cell stage. (C) Larval stage. (D) Iodine stain of a fourth-stage larva (300 μm in length) demonstrates a short buccal cavity, nerve rings (green arrow) between two bulbs of the rhabditoid esophagus, reflexed ovary, presence of vulva (red arrow), and anal pore (blue arrow).
the environment, *H. gingivalis* has been isolated from horse manure and compost (36). This organism has been reported from all inhabited continents except Australia (37), and isolates recovered from geographically distant localities appear to be genetically similar (6). In the present case, the affected woman had not traveled overseas or had contact with horses in the year prior to her presentation. Infection was likely acquired locally, but this cannot be confirmed as the epidemiology of *H. gingivalis* in Australia is unknown, and there is no published Australian case to date.

The present case is the sixth infection of a human by a *Haliaccephalobus* sp. described in the literature since 1975 (Table 1). Previously published human cases have all involved immunocompetent individuals in North America. All cases were fatal, with granulomatous encephalitis, suggestive of high neurotropism during infection. Diagnoses were made postmortem, and no anthelmintic treatment had been given. CSFs were obtained ante-mortem in three cases, white cells ranged from neutrophil to lymphocyte predominance, and pleocytosis with raised eosinophil levels was seen. Granulomatous inflammation was not seen in the current case, possibly due to the use of etanercept, a tumor necrosis factor-alpha (TNF-α) inhibitor, combined with methotrexate (38).

To date, 27 other cases of infection in animals have been described, mainly in horses, with 4 survivors (18, 25, 26, 29). *Micronema deletrix* was used as a synonym for this species in the 20th century. One horse with brain granulomata survived following aggressive debulking surgery complemented by ivermectin treatment (25). Transmission through a manure-contaminated wound had been proposed as the route of infection for one case but was not proven by autopsy (8). To date, human cases have not shed light on the route of transmission. In the present case, histopathological examinations of other organs did not indicate any dissemination of the nematode beyond the central nervous system (CNS). In animals, *H. gingivalis* had been linked to oromaxillary

![FIG 2](http://jcm.asm.org/)

(A) Hematoxylin and eosin (H&E) stain of brain tissue (under ×100 magnification) demonstrates perivascular inflammation with predominant macrophages and lymphocytes surrounding *H. gingivalis* larvae. (B) Third-stage larvae stained with H&E under ×400 magnification show presence of premature genital primordium (black arrow) and bulb of esophagus (at right end of nematode). (C) Microscopy examination of agar plate culture (×400 magnification) shows *H. gingivalis* larvae and eggs in various stages of development.
### TABLE 1
Reported cases of *H. gingivalis* infections in humans between 1975 and 2014

<table>
<thead>
<tr>
<th>Yr</th>
<th>Demographics</th>
<th>Country</th>
<th>Clinical presentation</th>
<th>Premortem CSF findings</th>
<th>Identification of organism</th>
<th>Anthelmintic</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>Human, 5-year-old male, immunocompetent</td>
<td>Canada</td>
<td>Meningoencephalitis</td>
<td>8 days after fall into manure spreader with facial &amp; mandible injuries</td>
<td>CSF—lymphocytic pleocytosis; 300 cells, 50% lymphocytes, 50% macrophages</td>
<td>Autopsy; morphological diagnosis; spinal cord involvement</td>
<td>No</td>
<td>Death</td>
</tr>
<tr>
<td>1979</td>
<td>Human, 47-year-old male, immunocompetent</td>
<td>United States</td>
<td>Meningoencephalitis, brainstem signs</td>
<td>Not available</td>
<td>Autopsy; morphological diagnosis; brainstem involvement</td>
<td>No</td>
<td>Death</td>
<td>9</td>
</tr>
<tr>
<td>1981</td>
<td>Human, 54-year-old male, heavy alcohol use</td>
<td>United States</td>
<td>Decubitus ulcers over buttock, bilateral internuclearophthalmoplegia, normal brain scan</td>
<td>CSF—lymphocytic pleocytosis</td>
<td>Autopsy; morphological diagnosis; brain, heart, liver, kidney involvement</td>
<td>No</td>
<td>Death</td>
<td>10</td>
</tr>
<tr>
<td>2010</td>
<td>Human, 39-year-old female, immunocompetent</td>
<td>United States</td>
<td>Meningoencephalitis; initial MRI &amp; lumbar puncture normal, improved temporarily with cyclophosphamide/prednisolone; repeat MRI—bilateral ring enhancing lesions</td>
<td>CSF—lymphocytic pleocytosis</td>
<td>Autopsy; morphological diagnosis; brain involvement</td>
<td>No</td>
<td>Death</td>
<td>11</td>
</tr>
<tr>
<td>2013</td>
<td>Human, 65-year-old female, immunocompetent</td>
<td>United States</td>
<td>Blurring of vision, encephalopathy, fever, MRI unremarkable</td>
<td>CSF—PMN pleocytosis; 160 leukocytes, 35% macrophages, 27% eosinophils, 14% neutrophils, 20% lymphocytes</td>
<td>Autopsy; morphological diagnosis; brain involvement only</td>
<td>No</td>
<td>Death</td>
<td>12</td>
</tr>
<tr>
<td>2014</td>
<td>Human, 74-year-old female, immunocompetent</td>
<td>Australia</td>
<td>Meningoencephalitis with brainstem signs, MRI frontoparietal meningitis</td>
<td>CSF—pleocytosis; 2,500 neutrophils, 34 mononuclear cells</td>
<td>Autopsy; morphological diagnosis confirmed with LSU rDNA PCR and sequencing</td>
<td>No</td>
<td>Death</td>
<td>13</td>
</tr>
</tbody>
</table>

a CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PMN, polymorphonuclear leukocytes; LSU rDNA, large subunit ribosomal DNA.
<table>
<thead>
<tr>
<th>Yr</th>
<th>Clinical detail</th>
<th>Country</th>
<th>Presentation</th>
<th>Laboratory diagnosis</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Horse</td>
<td>United Kingdom</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>13</td>
</tr>
<tr>
<td>1990</td>
<td>Two horses</td>
<td>United States</td>
<td>Disseminated infection, lung infection, encephalitis, spinal cord lesions</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>14</td>
</tr>
<tr>
<td>1992</td>
<td>Horse</td>
<td>Scotland</td>
<td>Disseminated infection with encephalitis and renal abscess</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>15</td>
</tr>
<tr>
<td>1993</td>
<td>Horse</td>
<td>United States</td>
<td>Mandible osteomyelitis renal abscess encephalitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Ivermectin followed with fenbendazole; deterioration on therapy</td>
<td>Death</td>
<td>16</td>
</tr>
<tr>
<td>1993</td>
<td>Horse</td>
<td>United States</td>
<td>Maxillary sinus abscess encephalitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Fenbendazole for maxillary sinus infection; deterioration on therapy</td>
<td>Death</td>
<td>17</td>
</tr>
<tr>
<td>1993</td>
<td>Horse</td>
<td>United States</td>
<td>Osteomyelitis gingivitis</td>
<td>Morphology</td>
<td>Ivermectin and diethylcarbamazine</td>
<td>Survival</td>
<td>18</td>
</tr>
<tr>
<td>1993</td>
<td>Horse</td>
<td>United States</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>Fenbendazole, dimethyl sulfoxide, desamethasone, and butazolidine</td>
<td>Death</td>
<td>19</td>
</tr>
<tr>
<td>1995</td>
<td>Horse</td>
<td>United States</td>
<td>Posthitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Fenbendazole, dimethyl sulfoxide, desamethasone, and butazolidine</td>
<td>Survival</td>
<td>20</td>
</tr>
<tr>
<td>1998</td>
<td>Horse</td>
<td>Germany</td>
<td>Osteomyelitis gingivitis</td>
<td>Morphology</td>
<td>Ivermectin</td>
<td>Death</td>
<td>21</td>
</tr>
<tr>
<td>2000</td>
<td>Two horses</td>
<td>United States</td>
<td>Encephalitis uveitis nephritis</td>
<td>Retrospective; morphology</td>
<td>Ivermectin</td>
<td>Death</td>
<td>22</td>
</tr>
<tr>
<td>2000</td>
<td>Zebra</td>
<td>United States</td>
<td>Ocular infection</td>
<td>Prospective; morphology</td>
<td>Ivermectin</td>
<td>Death</td>
<td>23</td>
</tr>
<tr>
<td>2000</td>
<td>Horse</td>
<td>Canada</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>24</td>
</tr>
<tr>
<td>2001</td>
<td>Horse</td>
<td>United States</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>25</td>
</tr>
<tr>
<td>2001</td>
<td>Horse</td>
<td>Canada</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>26</td>
</tr>
<tr>
<td>2001</td>
<td>Horse</td>
<td>Canada</td>
<td>Encephalitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Ivermectin + surgical debulking of granulomas</td>
<td>Survival</td>
<td>27</td>
</tr>
<tr>
<td>2004</td>
<td>Donkey</td>
<td>United States</td>
<td>Renal abscess</td>
<td>Prospective; morphological diagnosis</td>
<td>Ivermectin</td>
<td>Survival</td>
<td>28</td>
</tr>
<tr>
<td>2006</td>
<td>Horse</td>
<td>United States</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>29</td>
</tr>
<tr>
<td>2007</td>
<td>Horse</td>
<td>Japan</td>
<td>Encephalitis</td>
<td>LSU rDNA PCR and sequencing</td>
<td>No</td>
<td>Death</td>
<td>30</td>
</tr>
<tr>
<td>2007</td>
<td>Horse</td>
<td>Switzerland</td>
<td>Posthitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Prednisolone + topical moxidectin + oral moxidectin for 3 mo</td>
<td>Survival</td>
<td>31</td>
</tr>
<tr>
<td>2008</td>
<td>Horse</td>
<td>Canada</td>
<td>Mandibular abscess encephalitis</td>
<td>Prospective; morphological diagnosis</td>
<td>Progression on ivermectin, changed to thiabendazole</td>
<td>Death</td>
<td>32</td>
</tr>
<tr>
<td>2011</td>
<td>Horse</td>
<td>United Kingdom</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>33</td>
</tr>
<tr>
<td>2012</td>
<td>Horse</td>
<td>Canada</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>34</td>
</tr>
<tr>
<td>2012</td>
<td>Two horses</td>
<td>Iceland</td>
<td>Encephalitis</td>
<td>Autopsy; morphological diagnosis</td>
<td>No</td>
<td>Death</td>
<td>35</td>
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<tr>
<td>2014</td>
<td>Horse</td>
<td>South Korea</td>
<td>Encephalitis</td>
<td>LSU rDNA PCR and sequencing</td>
<td>Unspecified anthelminths</td>
<td>Death</td>
<td>36</td>
</tr>
</tbody>
</table>

*LSU rDNA, large subunit ribosomal DNA.*
infections and posthithis, suggestive of initial mucosal exposure to 
the invasive larvae, followed by dissemination (17, 18, 20, 29, 30). 
Exposure through the oromaxillary route may explain the com-
mon neurological involvement.

All human cases of H. gingivalis infections reported to date 
were diagnosed at autopsy, despite antemortem suspicions of 
parasitic infection in some cases. Neurological nematodiasis is rare 
but can involve parasites such as Angiostrongylus cantonensis, 
Strongyloides stercoralis, Toxocara canis, Trichinella spiralis, and 
Gnathostoma spinigerum, typically associated with CSF and per-
ipheral eosinophilia (39–46). During H. gingivalis infection, CSF 
may initially show only moderate pleocytosis and eosinophilia 
may be absent (10, 11) and larvae are usually not found in CSF 
obtained by lumbar puncture. To our knowledge, there is cur-
rently no immunossay or PCR readily available for H. gingivalis 
to provide a timely diagnosis. The D2 and D3 domains of LSU 
rDNA might be suitable targets for development of a real-time 
diagnostic PCR (6). The preliminary diagnosis of H. gingivalis can 
be made from nematodes obtained at autopsy since (i) the eggs are 
distinctive being thin shelled, elongate, and oval at various stages 
of development, including mature larvae, and (ii) although the larvae 
have a rhabditiform esophagus and superficially resemble the 
rhabditiform larvae of S. stercoralis, H. gingivalis has two 
ophalgeal bulbs whereas S. stercoralis has one, and the esophageal 
neck and buccal capsule are longer in H. gingivalis. In addition, 
only filiform larvae of S. stercoralis, which have a cylindrical 
esophagus and a notched tail, have been found in the CNS in 
disseminated strongyloidiasis (47) and (iii) if adult nematodes are 
found, they are female only with distinctive morphology.

Treatment responses can be assessed only from previous cases 
in animals, as none of the human cases, including this case, re-
ceived anthelmintics. Most affected animals deteriorated (16, 17, 
19, 21, 22, 30) despite treatment, and the presence of live worms at 
autopsy suggests that the anthelmintic treatment was ineffective. 
In vitro susceptibility testing using microagar larval developmen-
tal tests (MALDTs) has been used to assess the effects of thia-
bendazole and ivermectin on the hatching rate and larval devel-
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24. 27.

26. 1774

Spalding MG, Greiner EC, Green SL.

14. Case Report


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granuloma caused by


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