Fatal Human Meningoencephalitis due to *Halicephalobus* Nematodes, Germany

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Infections with *Halicephalobus* nematodes, causative agents of severe meningoencephalitis in horses, have rarely been reported in humans. In this study, the clinical, serological, cytokine, and histopathological findings of a rapidly progressive and eventually fatal meningoencephalitis in a previously healthy human are described. The helminth was finally diagnosed by specific polymerase chain reactions from post mortem tissue.

Keywords. *Halicephalobus*; horses; meningoencephalitis; nematode; PCR.

Halicephalobus gingivalis (formerly *Micronema deletrix*) is a free-living saprophagous nematode that lives in soil, decaying humus, and manure. The organism causes lethal meningoencephalitis and disseminated disease in horses worldwide [1–3]. So far, 5 human cases have been reported, all fatal infections of the central nervous system (CNS) in North America [3–7]: 1 in Canada [4] and 4 in the United States [3, 5–7]. All diagnoses have been established histopathologically post mortem. The parasite has also been detected in lungs, liver, and heart in human cases [1, 4, 5]. The nematode is parthenogenetic, and infected tissues may contain adults, larvae, and eggs [1]. Human infections have been linked to skin injuries and contact with soil or manure [4–6].

In this study, we describe a fatal human case in Germany diagnosed by histopathology and specifically designed polymerase chain reaction (PCR) tests from post mortem brain tissue and cerebrospinal fluid (CSF). We performed molecular comparisons of this case with a previous histopathologically diagnosed case from the United States and describe serological cross-reactions and cytokine responses.

CASE REPORT

A 49-year-old German paper mill worker with an unremarkable medical history was admitted (Department of Neurology, University Hospital Würzburg, Würzburg, Germany) with acute confusion and fever of 40°C. General and neurologic exam were otherwise normal. Further history taking did not reveal any diagnostic hint, particularly no traveling history, animal contact, nor outdoor activities. Laboratory investigations revealed a slight leukocytosis (11.4 G/L) with normal differential white cell count. C-reactive protein (CRP) and procalcitonin levels were normal. A toxicology screening was negative. Computed cranial tomography (CCT) showed slight brain edema. Cerebrospinal fluid analysis revealed a pleocytosis of 345 cells/μL with 90% lymphocytes (activated B- and T-lymphocytes, scattered plasma cells), few monocytes, and elevated protein concentration. Cerebrospinal fluid glucose level was normal. The constellation was compatible with an aseptic (meningo-) encephalitis; ceftriaxone, ampicillin, and acyclovir were administered. However, the patient progressed to coma. There was no sign of a nonconvulsive status epilepticus in several electroencephalograms, and a follow-up CCT confirmed progressive brain edema. Abdominal sonography was normal. Repeat lumbar puncture on day 5 showed elevated intracranial pressure (25 cm H_2O) and a persistent pleocytosis (430 cells/μL; 70% lymphocytes, 20% neutrophils, and 7% eosinophils). Various cultures, molecular, serological, and autoimmune investigations from CSF and serum were negative. As CRP levels increased to 9.75 mg/dL, antibiotics were changed to meropenem and vancomycin, complemented by dexamethasone. The patient was intubated, and magnetic resonance imaging showed hyperintense lesions in all cerebral lobes and focally in the basal ganglia (Figure 1A). Neither parenchymal nor meningeal contrast enhancement nor signs of cerebral vein thrombosis were present; time-of-flight angiography was inconspicuous. High-dose methylprednisolone did not change the clinical condition. On day 11, acute bilateral mydriasis developed. Computed cranial tomography displayed profuse brain swelling, sulcal effacement,

Received 20 January 2015; accepted 28 April 2015.

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Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofv061

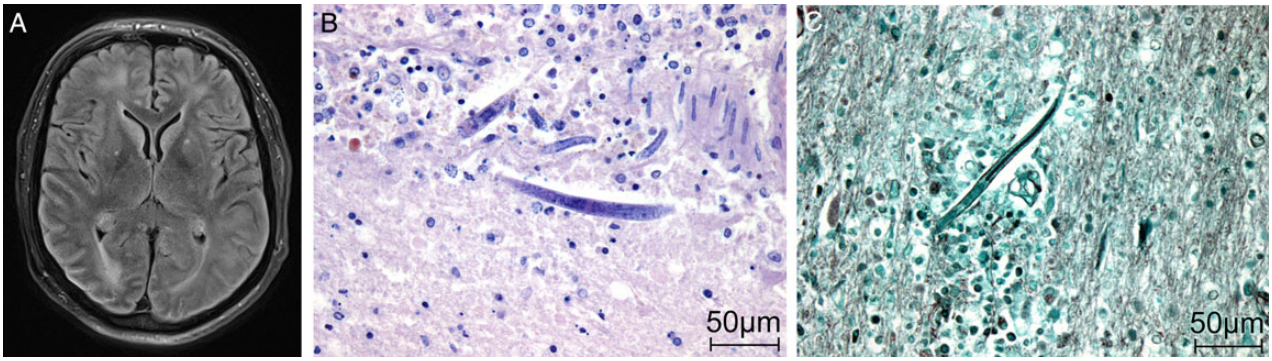

Figure 1. (A) Cranial magnetic resonance imaging. Axial FLAIR imaging with diffuse patchy hyperintense lesions and relative sparing of cortical U-fibers as a hint of unspecific brain edema. (B) Histopathology of brain lesions. Several nematodes are visible in different section planes. The small nuclei of the parasite are clearly discernable within the organisms. Hematoxylin and eosin stain. (C) Close-up view of a single nematode in longitudinal section. The darkly stained posterior digestive tract is easily discernable; a more delicately stained rhabditiform esophagus is visible at the anterior end of the helminth. The nematodes depicted here measured 150 × 15 µm, representing developing larval stages that underscore the parthenogenetic reproduction potential of *Halicephalobus*. Methenamine silver stain.

and midline shift despite decompressive hemicraniectomy. Death from transtentorial herniation with asystolia occurred the next day.

A limited, brain-only, post mortem examination revealed pronounced brain edema with restricted lateral ventricles, subfalcial herniation of the right enlarged hemisphere, and symmetrical cerebellar herniation. Microscopic examination demonstrated extensive inflammation in the cerebral parenchyma, ventricular system, meninges, upper brainstem, and cerebellum with perivascular and intramural lymphoplasmacytic infiltrates. The parenchymal infiltrates were composed of lymphocytes, plasma cells, monocytes or macrophages, and a few granulocytes, mostly eosinophils. The infiltrates were distributed over the white matter as either localized or diffuse, containing numerous nematodes (Figure 1B and 1C). The parasites had a small buccal cavity and a rhabditiform esophagus. No lateral alae or prominent cuticular ridges were present. Smaller and larger nematodes were visible. However, on the plane sections, the whole length of the organisms was only rarely seen. Smaller individuals measured 150 × 15 µm, and larger ones measured approximately 260–450 × 20 µm. From formalin-fixed, paraffin-embedded (FFPE) and frozen brain samples, panfilarial 12S rDNA- [8] and cytochrome oxidase gene-PCRs [9] were performed. The resulting amplicons were sequenced, but BLAST analysis (www.ncbi.nlm.hih.gov/blast) revealed only low similarities of 79%–93% to rDNA from various nematode genera. Thereupon, 2 PCRs were performed targeting the 28S rDNA of *Halicephalobus* nematodes [10]. The PCRs were positive from frozen brain tissue (98% identical nucleotides to deposited *Halicephalobus gingivalis* 28S rDNA sequence) but negative from FFPE samples and CSF. Two specific PCRs targeting small regions of the *Halicephalobus* 28S rDNA were thus designed. The resulting 117- and 249-basepair (bp) amplicons from frozen samples and FFPE tissue and the 117-bp amplicon from CSF were 100% identical to known *Halicephalobus gingivalis* sequences. In addition, the newly designed PCRs were successfully used on stored FFPE material from a North American case [6], with identical results (Supplementary Table 1). Only few nucleotide differences were detected in the alignments (Supplementary Figure 1). The 12S rDNA and cytochrome oxidase gene sequences of *Halicephalobus gingivalis* from the fatal case described herein have been submitted to GenBank (accession numbers KP347445 and KP347444, respectively). Based on the molecular data and morphology of the abundant nematodes, with the typical presence of adult and juvenile nematodes, cerebral halicephalobiasis was diagnosed.

Retrospective trichinellosis and toxocariasis immunoblot serology (BioRépair, Sinsheim, Germany) showed weak antibody reactions in serum but not in CSF. No serum antibodies were detected by in-house enzyme-linked immunosorbent assays for dirofilariosis, ascariasis, strongyloidiasis, and trichinosis. Cytokine analysis (Bio-Rad Laboratories, Munich) revealed highly elevated serum interleukin-5, -6, and -7 levels and increased monocyte chemoattractant protein (MCP)-1, RANTES, and vascular endothelial growth factor concentrations (49.17, 58.8, 17.06, and 126.0, 5533.23, 293.3 pg/mL, respectively; 10 healthy control sera, <2, <10, <12, and <100, <2800, <250, <2 pg/mL, respectively). There was no difference in levels for tumor necrosis factor-α, interferon-γ, interleukin-1, -2, -4, -8, -9, -10, -12, -13, -15, -17, and eotaxin between the patient and healthy controls.

DISCUSSION

In this study, we describe a fatal human meningoencephalitis by *Halicephalobus* outside North America. As in all previously

reported human infections, the diagnosis was established post mortem. The lack of ante mortem symptoms suggestive of an invasive helminth infection is typical for human halicephalobiasis [3–7]. Because there was no peripheral blood or initial CSF eosinophilia, and no visible helminths in the CSF either, anthelmintics were not administered. Only a few laboratory parameter changes were observed during the clinical course in our patient: a late rise of CRP, which was most probably due to a bronchopneumonia in the progressively comatose patient, and an elevated creatine kinase, most probably of brain origin. It remains uncertain whether the immunosuppressive treatment, also administered in another case [6], negatively impacted the clinical course. In all of the previous human cases, nematodes were not seen in the CSF. Retrospectively, weak serological cross-reactions to nematode antigens were demonstrated in serum but not in CSF. Post mortem histopathology revealed only few parasites in the meninges. In contrast, many parasites were detected in the brain parenchyma, and these were surrounded by an inflammatory infiltrate with few eosinophils. Infrequent or rare eosinophil infiltration was also noted in 4 previous human cases [3–5, 7]. Lymphocytic pleocytosis was described in 3 patients [4–6], but elevated eosinophil counts in CSF were only reported once [7]. In the latter case (the only with reported full blood count), peripheral blood eosinophilia was absent. Although our retrospective analyses showed elevations of RANTES, MCP-1, and interleukin-5 levels (which usually induce eosinophilia, mast cell recruitment, and B-cell proliferation [11, 12]), strikingly few tissue eosinophils and normal levels of eotaxin were present. It remains speculative whether an unknown underlying immune defect predisposes humans for infections with this parasite or whether the parasite actively downregulates eosinophil recruitment.

The final diagnosis was based on molecular detection of *Halicephalobus* DNA from brain tissue using protocols established for equine cases [10] and newly developed short-fragment PCRs described herein. Using the latter PCRs, a histological comparison case from the United States [6] was molecularly confirmed as halicephalobiasis. Contamination of wounds and penetration of mucus membranes or the skin is the most likely port of entry [4–7]. In the present case, contact with contaminated wood or waste paper that was recycled in the paper mill might have been the source of infection. In horses, hematogenous spread and tissue migration of the nematodes has been demonstrated [2]. For human and equine CNS and disseminated halicephalobiasis, no established treatment exists, and the nematodes have shown a certain degree of tolerance to ivermectin and benzimidazoles [6, 13, 14]. In summary, this case illustrates the clinical course and diagnostic challenges of an unusual and sudden infection in a previously healthy individual.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

Acknowledgments

Potential conflicts of interest. All authors: No reported conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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