History

A young adult 12-kg (26.4-lb) female mixed-breed dog housed in an animal shelter was referred to the teaching hospital of the Department of Veterinary Medical Sciences of the University of Bologna because of severe nonambulatory tetraparesis and abnormal mentation. The dog was found wandering 2 months before; at that time, the dog had mild paraparesis that slowly progressed to tetraparesis and recumbency. In the 2 weeks preceding the referral examination, additional clinical signs included obtunded mental status and intention tremors. Prednisone (1 mg/kg [0.45 mg/lb], PO, q 24 h for 6 days) was administered to the dog without any improvement.

Clinical and Clinicopathologic Findings

General physical examination findings were unremarkable, except for neurologic abnormalities. The dog was in lateral recumbency with severe nonambulatory tetraparesis, obtunded mental status, left-sided head tilt, occasional tremors of the neck and head, and spontaneous proprioceptive deficits in all 4 limbs. Cranial nerve examination revealed vertical spontaneous nystagmus and an abnormal menace response. History and clinical signs were consistent with a suspected multifocal neurologic disorder, primarily involving the brainstem. Results of routine hematologic evaluation and serum biochemical analysis were within references ranges. A sample of CSF was collected via cisternal puncture and analysis revealed marked abnormalities (Table 1). Magnetic resonance imaging was not performed because of financial constraints.

Table 1—Cerebrospinal fluid data for a young mixed-breed dog that was evaluated because of abnormal mentation and progressive nonambulatory tetraparesis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/dL)</td>
<td>640.1</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>285.45</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Cell count (cells/µL)</td>
<td>680</td>
<td>0–5</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>6</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>11</td>
<td>60–70</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>82</td>
<td>30–40</td>
</tr>
</tbody>
</table>

This report was submitted by Luciana Mandrioli, DVM, PhD; Antonella Gallucci, DVM, PhD; Filippo Scarpa, DVM, PhD; Chiara Brachelente, DVM, PhD; and Gualtiero Gandini, DVM, PhD; from the Department of Veterinary Medical Sciences, School of Agriculture and Veterinary Medicine, University of Bologna, 40064 Ozzano Emilia, Bologna, Italy (Mandrioli, Gallucci, Scarpa, Gandini); and the Department of Biopathological Science and Hygiene of Food and Animal Production, Faculty of Veterinary Medicine, University of Perugia, 06126 Perugia, Italy (Brachelente). Dr. Scarpa’s present address is CDVet Via Francesco Ferratoni 94c, 00177 Rome, Italy.

Address correspondence to Dr. Mandrioli (luciana.mandrioli@unibo.it).
Additional Laboratory Testing

Serum and CSF samples were tested for antibodies against *Toxoplasma* spp and *Neospora* spp. Both samples were negative for *Toxoplasma* spp but strongly positive for *Neospora* spp (in both samples, the IgG titer was 1:640 (reference interval applied to both serum and CSF, < 1:40). A PCR assay was performed on the CSF sample, and the result was positive for *Neospora caninum*. Despite treatment with clindamycin (20 mg/kg [9.09 mg/lb], IV, q 12 h) and IV fluid therapy, the dog’s condition worsened and it died 2 days after the initial referral examination. Postmortem examination of the CNS was performed.

Postmortem Findings

Postmortem examination did not reveal any macroscopic changes in the CNS or major organs. Microscopic examination of sections of the brain (cerebral cortex) revealed that neuroparenchyma was affected by multifocal malacia and reactive gliosis associated with intralesional groups of protozoa; numerous deeply basophilic bradyzoites measuring 6.5 × 1.5 µm were present, often within the cytoplasm of glial cells (Figure 1). There was evidence of nonsuppurative lymphoplasmacytic meningoencephalitis and reactive gliosis; sometimes, groups of bradyzoites were found adjacent to capillaries and within the neuroparenchyma.

Samples of the cerebellum and brainstem contained scattered cysts without septa, with a wall thickness of 0.5 to 4 µm (Figure 1); a concurrent loss of Purkinje and granular cells was found in the cerebellum, along with marked inflammation of the folia. Severe demyelination had occurred focally in the fiber tracts, especially in the cervical portion of the spinal cord and included swollen axon sheaths.

Ultrastructural examination of brain and cerebellum revealed bradyzoites grouped together and not enclosed by a cyst wall; intact bradyzoites had an apical part (conoid) rich in micronemes, rhoptries with an electron-dense content, and reactive gliosis associated with intralesional groups of protozoa; numerous deeply basophilic bradyzoites measuring 6.5 × 1.5 µm were present, often within the cytoplasm of glial cells (Figure 1). There was evidence of nonsuppurative lymphoplasmacytic meningoencephalitis and reactive gliosis; sometimes, groups of bradyzoites were found adjacent to capillaries and within the neuroparenchyma.

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dense content, electron-lucent amylopectin granules, and a nucleus (Figure 1), supporting the etiologic diagnosis of neosporosis.

**Morphologic Diagnosis and Case Summary**

Morphologic diagnosis: severe, chronic, lymphoplasmacytic and histiocytic meningoencephalitis (brainstem) and cerebellitis, with multifocal malacia, reactive gliosis, intrasional bradyzoites, and cysts consistent with protozoal organisms (*N. caninum*).

Case summary: meningoencephalitis and cerebellitis due to *N. caninum* infection in a young adult dog with progressive cerebellar signs.

**Comments**

For the dog of the present report, findings of neurologic and CSF examinations were consistent with an intracranial infectious or inflammatory disease. Examination of the CSF sample revealed severe mononuclear pleocytosis, and differential diagnoses of infectious meningoencephalitis caused by protozoa and noninfectious inflammatory disease (eg, meningoencephalitis of unknown etiology) were considered. Although mononuclear, neutrophilic, or mixed pleocytosis with moderately or markedly high protein concentration has been associated with both of those diseases, in cases of protozoal meningoencephalitis, the CSF most often has characteristics suggestive of mild to moderate inflammation, with a mixed population of neutrophils and mononuclear cells and occasional eosinophils. In meningoencephalitis of unknown etiology, examination of CSF samples typically reveals mononuclear pleocytosis, which is variable in severity.

In the case described in this report, the presence of intrasional parasites was strongly indicative of neosporosis, although toxoplasmosis could also have been considered. Appropriate PCR assays and immunohistochemical analyses of CSF samples can be very helpful in achieving a definitive diagnosis.

*Neospora caninum* is a primary pathogen in dogs and can cause clinical disease in dogs of all ages. Most clinical cases of canine neosporosis have involved congenitally infected littermates. Hind limb paresis that develops into progressive paralysis, often associated with muscular contracture and fibrosis, is the most consistent sign of neonatal neosporosis in dogs. Signs of disease are seen most frequently in young or immunocompromised dogs, but a wide array of clinical signs in older dogs has also been reported. Adult dogs may develop neosporosis either as a result of a new infection or through reactivation of a latent infection. Pathogenesis of continuous multiplication of *N. caninum* tachyzoites in chronically infected dogs is intriguing and may be associated with cyst rupture. In those dogs, chronic and progressive multifocal neurologic signs predominate and consistent involvement of cerebellum has been described. Even though there are several reports of successful treatment of adults and neonates with neosporosis, the prognosis of neosporosis involving the nervous system is generally considered guarded and dependent on the duration and clinical course of the disease. Moreover, the treatment of clinical neosporosis in dogs with currently available drugs, including clindamycin, is only partially effective because of permanent damage caused by the organism and possibility of relapse.

The reason for the tendency of *N. caninum* to cause cerebellar dysfunction is not currently clear; among the adult canine cases of cerebellar disease, numerous protozoa were found in the cerebellum, although the number of parasites was low in other instances, despite the presence of severe malacia of the cerebellar molecular layer. These findings suggest that the tissue response does not necessarily correlate with parasite burden and raises the possibility of an immunologic component to the condition.

In the dog of the present report, the morphologic peculiarities of the bradyzoites, such as rhoptries, micronemes, and amylopectin granules identified by ultrastructural examination, contributed to the their characterization as belonging to the genus *Neospora*, considering that these organelles are specific constituents of these protozoa. The morphological findings reinforced the molecular testing results and differentiated the dog’s condition from an infection attributable to *Toxoplasma spp.*

**References**