

# Pathology in Practice

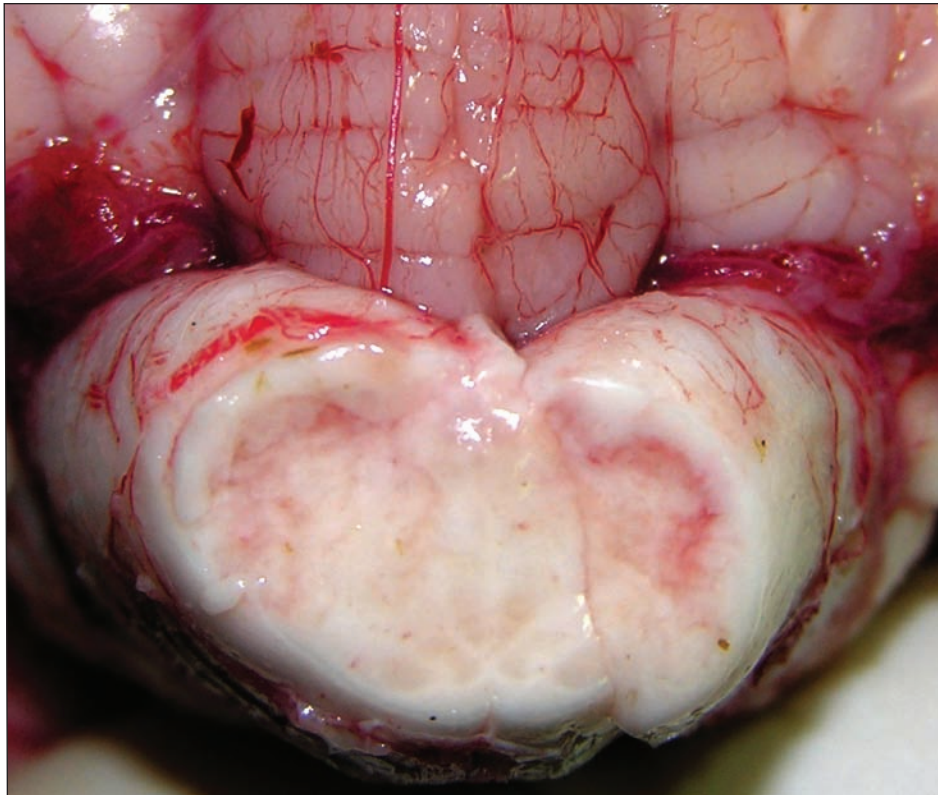


Figure 1—Photograph of a cross section of the medulla oblongata of a 5-month-old Nubian-Boer goat that was found laterally recumbent with ventromedial strabismus approximately 6 hours prior to death. Notice that the parenchyma is soft (malacia).

## History

A 5-month-old Nubian-Boer goat in good body condition from a small backyard herd comprised of 5 goats was submitted for necropsy. According to the submission information, the goat was found laterally recumbent with ventromedial strabismus approximately 6 hours prior to death. No other animals in the group were affected. In addition, 2 tubes of whole blood col-

lected from the goat before death were submitted for an agar-gel immunodiffusion test for circulating antibodies against caprine arthritis-encephalitis virus. No other history was provided.

## Clinical and Gross Findings

On gross examination, the goat's oral mucous membranes were moderately pale. Moderate numbers of *Haemonchus contortus* were found in the abomasum. A focally extensive, slightly hemorrhagic, depressed, and softened area was observed in the medulla oblongata close to the obex (Figure 1). Multiple samples of the brain were submitted for testing with fluorescent antibodies against rabies virus and *Listeria* organisms. Feces from the rectum were collected for routine fecal flotation examination.

Formulate differential diagnosis from the history, clinical findings, and Figure 1—then turn the page →

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## Histopathologic Findings

Tissue samples from the goat's brain were processed for histologic examination. The brainstem (medulla oblongata through the midbrain) and cranial cervical spinal cord contained perivascular cuffs composed of multiple layers of lymphocytes, plasma cells, macrophages, and occasional neutrophils (Figure 2). The inflammatory infiltrate extended into the parenchyma, forming small nodules, and into the leptomeninges. Multiple neurons entrapped within the inflammatory nidus were round, pale, and eosinophilic, each with loss of Nissl substance and without a nucleus (neuronal necrosis). Occasionally, degenerate neurons were surrounded by glial cells. Axons were swollen and commonly replaced by macrophages (digestion chambers). Foamy macrophages (gitter cells) were also evident throughout the parenchyma. The trigeminal nerve contained multiple small clusters of moderate numbers of lymphocytes and macrophages and rare neutrophils. A few gram-positive rods (detected by use of Lilly-Twort Gram stain) were present within the cytoplasm of macrophages in the brainstem and trigeminal nerve. Immunohistochemical analysis for *Listeria monocytogenes* revealed a few bacteria within cytoplasm of the inflammatory cells in the brainstem and trigeminal nerve.

## Ancillary Laboratory Test Results

Although results of immunohistochemical analysis were positive for *L monocytogenes* in this goat, fluorescent antibody testing for the organism yielded negative results. Testing of brain tissue samples with fluorescent antibody against rabies virus and testing of whole blood samples with an agar-gel immunodiffusion assay for antibodies against caprine arthritis-encephalitis virus also yielded negative results. The fe-

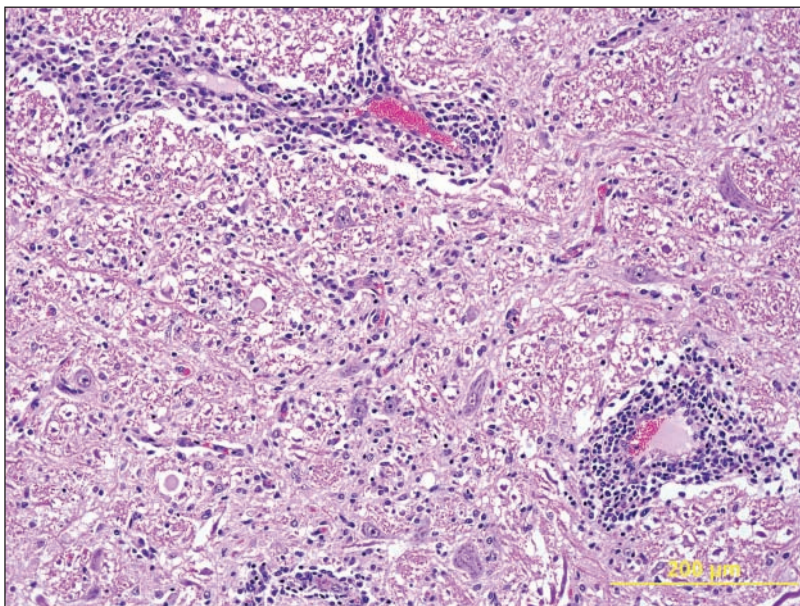


Figure 2—Photomicrograph of a section of brainstem of the goat in Figure 1. Multiple thick perivascular cuffs composed of lymphocytes, plasma cells, and fewer macrophages are visible in this section. The inflammatory infiltrate extends into neuropil and neuroparenchyma. A few neurons are degenerated, and occasional axonal spheroids are seen. H&E stain; bar = 200  $\mu$ m.

ces collected from the goat of the present report contained 100 trichostrongyle eggs/g and low to moderate numbers of *Eimeria* spp.

## Morphologic Diagnosis

Encephalitic listeriosis with severe lymphohistiocytic rhombencephalitis and leptomeningitis of the brainstem and multifocal mild lymphohistiocytic neuritis of the trigeminal nerve with intracellular gram-positive rod bacteria.

## Comments

The encephalitic form of listeriosis was diagnosed in the goat of the present report on the basis of the lymphohistiocytic rhombencephalitis (inflammation of the brainstem) and neuritis as well as the presence of bacteria, which had a positive immunohistochemical reaction with antibody against *L monocytogenes*, within the cytoplasm of mononuclear cells in the trigeminal nerve. Listeriosis (also known as silage or circling disease) is most commonly caused by *L monocytogenes*, a gram-positive, nonspore-forming, facultative anaerobic intracellular coccobacillus.<sup>1</sup> There are 6 known species of *Listeria*, and only 2 are considered pathogenic: *L monocytogenes* and *Listeria ivanovii*. *Listeria* spp have many mammalian hosts, including domestic ruminants (goats, sheep, and cattle), horses, dogs, cats, pigs, deer, and humans.<sup>1</sup> There are 3 main syndromes associated with listeriosis: encephalitis, abortion, and septicemia.<sup>2</sup> Ruminants, especially goats, are highly susceptible to listeriosis and are typically infected via the oral route.<sup>1,3</sup>

The clinical signs associated with encephalitic listeriosis in ruminants are initially nonspecific, including fever and signs of depression. Within 1 to 3 days after infection, affected patients typically develop unilateral or bilateral signs attributable to brainstem and cranial nerve lesions.<sup>2</sup> As neural involvement worsens, signs become more severe and include dullness, torticollis, ipsilateral circling, unilateral facial paralysis, and drooling secondary to pharyngeal paralysis. Unilateral asymmetric cranial nerve deficits are most commonly reported. Unfortunately, despite antimicrobial treatment, death usually occurs within a few days after the initial signs develop; prior to death, there is generally a rapid progression to recumbence, head pressing, paddling of the limbs, and convulsions. In sheep and goats, compared with cattle, the disease is more acute and mortality rate is greater.<sup>1</sup>

Clinical pathological evaluation can help to rule in listeriosis but will not be able to exclude it as a differential diagnosis. For affected animals, a CBC may reveal no abnormalities or may indicate neutrophilic leukocytosis; a sample of CSF may appear cloudy and have high total protein concentration and cell counts. The key feature is that despite

the fact that listeriosis is a bacterial disease, mononuclear cells (hence the name *L monocytogenes*) predominate in the CSF in most cases. Bacteria are rarely observed in CSF samples from affected animals, and bacterial culture of CSF samples is usually unrewarding.<sup>4</sup> Overall, results of CSF analysis can help rule out other differential diagnoses like aberrant parasite migration. However, a definitive antemortem diagnosis is difficult because of the nonspecific clinical signs and rapid progression of the disease.<sup>5</sup>

In animals with listeriosis, gross lesions are generally absent. However, small areas of softening (malacia) can sometimes be detected in the brainstem, especially in the area of cranial nerves V, VII, VIII, and X.<sup>5</sup> Collected samples of CSF are often cloudy. Microscopically, multifocal asymmetric brainstem microabscesses and necrosis in the medulla, pons, and cerebellum are common. In addition, perivascular lymphohistiocytic cuffing with gliosis and a mononuclear infiltrate of meninges may be seen.<sup>1,2</sup> To make a definitive diagnosis of listeriosis, fresh brain tissue should be submitted for bacterial culture or for testing by use of other techniques such as immunohistochemical analysis, fluorescent antibody testing, and PCR assay.

The key to the success of *Listeria* spp (*L monocytogenes* and *L ivanovii*) as pathogens is the organism's ability to survive in hostile environments. Bacteria of this genus can resist environmental extremes in acidity and temperature, which allows organisms to replicate intracellularly in both phagocytes and nonphagocytes.<sup>2</sup> The manner in which *L monocytogenes* becomes disseminated is a matter of debate, but the predominance of lesions in the brain and cranial nerves indicates that both hematogenous as well as centripetal movement up neurons are possible routes of spread.<sup>2</sup> Neural-ascending infection has been established on the basis of evidence that *Listeria* organisms invade through the mucosa of the oral cavity and into the sensory and motor branches of the trigeminal nerve (cranial nerve V) as well as other cranial nerve branches.<sup>2</sup> *Listeria monocytogenes* also produces listeriolysin, a hemolysin, which is a virulence factor required for intracellular multiplication because it allows these bacteria to escape from the phagosome and enter the cytoplasm.<sup>6</sup> Additionally, surface proteins called internalins interact with E-cadherin, an intracellular adhesion molecule, to allow bacteria to invade virtually all host cells without being phagocytized.<sup>2,6</sup> Once in the CNS, the bacteria spread from cell to cell via the surface protein actA, which activates actin filaments in 1 pole of the bacterial organisms and propels them to

the periphery of the host cell. The bacteria use these mechanisms to travel from cell to cell within the brain, without exposure to the external environment and the host immune system.<sup>2,7</sup> In the neurologic form of the disease, this intracellular movement results in axonal injury and ultimately in neuronal death.<sup>7</sup>

The most common risk factor for listeriosis is the consumption of poorly preserved, incompletely fermented silage. Such forage will generally have a pH > 5.5.<sup>2</sup> Factors that limit the fermentation of silage include longer fiber length, decreased moisture, inadequate packing, no covers, and contamination with soil or feces. The mechanism for the association between incompletely fermented silage and listeriosis is not completely understood but may involve an acquired susceptibility of animals fed silage to infection or the provision of a growth medium in the incompletely fermented silage that allows for heavy organismal growth.<sup>2,3</sup>

Antimicrobial treatment of animals with listeriosis is often unsuccessful because of the organisms' ability to live intracellularly, along with the poor cellular penetration of most antimicrobials. Common antimicrobials used in the treatment of listeriosis are penicillin and oxytetracycline.<sup>5</sup> Among ruminants, prevention of disease can be attempted by properly storing silage (ensuring that fecal and soil contamination is limited) and minimizing stressors, such as transport.

## References

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