BRIEF COMMUNICATIONS and CASE REPORTS

Rabies-induced Spongiform Change and Encephalitis in a Heifer

G. L. Foley and J. F. Zachary

Abstract. A 1-year-old mixed breed heifer was presented to the Veterinary Medical Teaching Hospital at the University of Illinois with a 3-day history of abnormal mentation and aggressive behavior. Based on the history and clinical examination, euthanasia and necropsy were recommended. The differential diagnoses included rabies, pseudorabies, and a brain abscess. The brain was removed within 60 minutes of death, and the section submitted for fluorescent antibody testing was positive for rabies virus antigen. Residual brain tissue was immersion fixed in 10% neutral buffered formalin. Histologic examination revealed a marked perivascular and meningeal lymphocytic meningoencephalitis and locally extensive spongiform change of the gray matter affecting the neuropil and neuron cell bodies. The most severely affected regions with spongiform change were the thalamus and cerebral cortex. No Negri bodies were found in any sections. Since the outbreak of bovine spongiform encephalopathy (BSE) in the United Kingdom, there has been an increased surveillance of bovine neurologic cases in an effort to assess if BSE has occurred in the USA. In areas where rabies virus is endemic, rabies should be included as a possible differential diagnosis in cases of spongiform changes of the central nervous system.

Key words: Brain; cattle; rabies; spongiform encephalopathy; thalamus; vacuolation; virus.

With the occurrence of bovine spongiform encephalopathy (BSE) in the United Kingdom, a surveillance program has been established in the United States to monitor spongiform lesions in the central nervous system of domestic cattle. Spongiform changes in the medulla oblongata are considered pathognomonic for BSE and have been widely reported in the veterinary literature. In a recent article on the diagnostic characteristics of BSE, a number of causes of spongiform change were reviewed. Although the authors cited toxic, metabolic, and artifactual causes of spongiform changes in cattle, they did not include the potential for rabies to induce spongiform change. A recent series of articles has outlined the development of rabies-induced spongiform change in skunks and foxes. In this article, we review the case history and lesions of spongiform change and encephalitis in a 1-year-old heifer.

A 1-year-old mixed-breed beef heifer was presented to the Veterinary Medical Teaching Hospital at the University of Illinois with a 3-day history of progressive central nervous signs. Initially, the heifer appeared to manifest estrus behavior. Subsequently, the heifer did not eat or drink, became belligerent, attacked buckets, and began chewing on metal fences and on herself. On presentation, the heifer was unable to rise. Based on the rapid progression of nervous signs and the clinical presentation, differential diagnoses included rabies, pseudorabies, and an abscess within the brain. The heifer was euthanized with an intravenous injection of pentobarbital and potassium chloride and submitted for necropsy.

Because rabies was a strong differential diagnosis in this case, a complete necropsy was delayed pending results of rabies testing. Within 60 minutes of death, the head was removed and a longitudinal section of the brain, including hippocampus and cerebellum, was submitted for fluorescent antibody (FA) testing for rabies virus. The residual sections of the brain were immersion fixed in 10% neutral buffered formalin (NBF).

The FA test for rabies was positive, so the carcass was not necropsied. After 48 hours of fixation, the brain was cut in 1–2-cm transverse sections and allowed to fix in NBF for another 24 hours. Samples for histopathology were collected from the following sites: cerebrum (anterior, central, and posterior), brain stem (level of anterior fornix, level of optic chiasm, level of the thalamus), pituitary, cerebellum with brain stem, and medulla oblongata/spinal cord. Tissues were trimmed for processing and held in NBF another 24 hours to ensure all tissues were completely fixed to kill the virus. Samples were routinely processed, embedded in paraffin, and sectioned at 4 μm for hematoxylin and eosin (HE) staining.

Histologically, the two most striking lesions were the perivascular inflammation and a variable amount of spongiform change of the gray matter. There was marked inflammatory infiltrate around the vasculature and meninges that consisted primarily of lymphocytes. Perivascular spaces were dilated. There was a variable amount of spongiform change in the gray matter of different regions of the brain. The most severely affected areas with spongiform change were the thalamus (Fig. 1) and cerebral cortex. Less severely affected sites were scattered in the gray matter of the brain stem and medulla oblongata. Vacuoles were primarily in the neuropil but also occurred in the perikaryon of neurons. The vacuoles ranged in size from 4 to 40 μm in diameter and were round to oval with well-demarcated borders (Fig. 2). In the cerebral cortex, the vacuoles tended to be in the deeper layers of the
Fig. 1. Thalamus; heifer. There is with a locally extensive spongiform change of the neuropil and marked perivascular inflammatory infiltrates that vary in intensity in different areas of the brain. HE. Bar = 100 μm.

Fig. 2. Thalamus; heifer. Higher magnification of vacuoles located primarily in the neuropil. In some areas, vacuoles are also within the perikaryon of neurons. Some vacuoles are clustered and appear to coalesce. HE. Bar = 30 μm.

gray matter. In the thalamus, the vacuoles were scattered within the entire gray matter region. In spite of extensive searching, no Negri bodies were seen in any of the sections. Spongiform changes in ruminants can be the result of systemic diseases, toxicities, processing artifacts, and the "slow viruses" (scrapie in sheep and BSE in cattle). In the United Kingdom, the combination of a history of nervous signs and spongiform change in the brain of a cow are considered pathognomonic for BSE.

Spongiform change can also be induced by rabies virus infection. Rabies-induced spongiform change has been most extensively studied in experimentally infected skunks and foxes. The vacuoles induced by rabies occur primarily in the neuropil and occasionally in the neuronal perikarya. The two main sites of spongiform change in skunks are the thalamus and cerebral cortex, similar to our findings in this heifer. The inflammatory lesion of rabies is characterized as a lymphocytic infiltrate around the vasculature and in the meninges, with variable amounts of gliosis. The occurrence of Negri bodies can be variable. Similar spongiform changes have been briefly mentioned in spontaneous cases of rabies in the skunk, fox, horse, cow, and cat. The pathogenesis of the rabies-induced spongiform change is unknown but does not require the presence of viral antigen at the site of the lesion.

The lesion of BSE is typically described as a noninflammatory microcavitation of the gray matter affecting the neuropil and occasionally the neuronal perikarya. Additionally, the spongiform change is bilaterally symmetrical. The spongiform change of BSE is most prominent in the reticular formation of the medulla oblongata, the central gray matter in the midbrain, and the thalamus, with the medulla oblongata at the obex being the preferred section for diagnosis of BSE.

In a study comparing the lesions of scrapie and rabies in skunks, the spongiform changes were markedly similar in both diseases. The rabies-induced spongiform change tended to be less extensive and had larger vacuoles (2–60 μm for rabies, 2–25 μm for BSE) in the neuropil than did the scrapie-induced lesion. Additionally, rabies was accompanied by an inflammatory infiltrate and Negri bodies, although in clinical cases of rabies the inflammatory component is variable. Rabies-induced spongiform change can be distinguished from that of BSE based on the perivascular inflammatory component, distribution of vacuoles primarily in the thalamus and cerebral cortex, and tendency for larger vacuoles. Incubation periods and morbidity periods are typically shorter in rabies than in the scrapie-like disorders.

The diagnosis of rabies in this heifer was based on the rapid onset and short duration of clinical signs, marked ag-
gressive behavior, positive FA for rabies virus antigen, and the histologic lesion consisting of a lymphocytic meningoencephalitis concurrent with a spongiform change, similar in distribution to that previously reported in rabies-infected skunks. The source of the rabies infection in this heifer was not established. The skunk is the most likely source of rabies virus infection of domestic animals in Illinois (Illinois Department of Public Health, personal communication).

Many of the articles describing lesions of BSE come from the United Kingdom, so rabies is typically not a differential diagnosis in these cases. In the United States and other countries where rabies virus is endemic, cattle with histories of neurologic signs may be rabid. Additionally, the degree of inflammation or presence of Negri bodies is not consistent in all cases of rabies. Thus, the distinction between spongiform changes induced by BSE and those induced by rabies virus may not always be clear. Neurologic cases in cattle should be monitored for the occurrence of BSE. Rabies should also be considered as a possible differential diagnosis for spongiform changes in cattle and other domestic species.

References

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Amyloidosis in the Bottlenose Dolphin, *Tursiops truncatus*

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Abstract. Four cases of amyloidosis were recognized in a study population of 21 (19%) bottlenose dolphins (*Tursiops truncatus*) examined as part of an investigation of the causes of cetacean strandings along the Texas Gulf Coast. Amyloid deposition was mainly and most prominently in the corticomedullary regions of the kidneys and less consistently in the vessels of the spleen, lung, and heart and around acini of the palatal salivary gland and the thyroid gland. Pretreatment of sections with permanganate and sulfuric acid greatly diminished Congo red staining, suggesting the dolphin amyloid is of the AA variety.

Key words: Amyloidosis; cetaceans; dolphins; stranding; *Tursiops truncatus*.

Amyloidosis is a disease characterized by the tissue deposition of autologous extracellular fibrillar proteins with particular tinctorial and ultrastructural properties and is well recognized in a wide variety of animals, including birds, reptiles, and mammals. "Amyloid" is the term applied to a group of chemically diverse proteins that display remarkable morphologic and histochemical uniformity. Although amyloid is generally easily recognized using hematoxylin and eosin (HE) and conventional light microscopy, certain histochemical methods enhance recognition, define the amyloid, and help to categorize it. Congo red is the most commonly used stain for amyloid. However, although its reaction is typical and reliable, it is not completely specific because it also stains elastic fibers and, depending on the care applied to staining, collagen bundles. When viewed using polarizing microscopy, amyloid stained with Congo red displays a particular apple green color, which can be resolved into blue and yellow by rotation of the plane of polarization. This color...