Idiopathic disseminated intracytoplasmic neuronal vacuolation in a newborn Holstein calf born in the USA

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Abstract. Histopathologic, immunohistochemical, and ultrastructural evaluations were made of a 6-day-old Holstein calf with severe vacuolation of the neuronal perikarya that was widely distributed throughout the central nervous system. No evidence of storage material within the vacuoles was revealed by histopathologic and ultrastructural examinations. Immunohistochemical and electron microscopic examinations were negative for protease-resistant prion protein and scrapie-associated fibrils, respectively. These results indicate that the clinical signs in this calf were not associated with transmissible spongiform encephalopathy. Neuronal vacuolation has not previously been documented in calves.

Transmissible spongiform encephalopathies (TSEs) of animals include scrapie of sheep and goats, transmissible mink encephalopathy (TME) of mink, chronic wasting disease (CWD) of deer and elk, and bovine spongiform encephalopathy (BSE) of cattle. The latter has also been observed in domestic cats and several wildlife species. Since the outbreak of BSE in the UK in 1985, there has been concern that the disease would occur in the USA. If this were to happen, loss to the cattle, sheep, and animal by-product industries would be catastrophic. Also, the concern for public health would be enormous. Because the initial diagnosis of BSE will most likely be based on histopathology, as it was in the case of BSE outbreak in the UK, it is important that veterinary pathologists provide an accurate, early diagnosis of the disease so that control and eradication measures can be implemented immediately.

The histologic characteristics of TSEs are spongiform change in neuropil and the presence of cytoplasmic vacuolation in neurons. However, such findings may be present in conditions other than TSEs. In animals, spongiform changes affecting the neuronal and neuronal cell bodies have been described in experimental and natural cases of rabies in skunks, foxes, and a heifer, in weak and ataxic Rottweiler pups, and in young neurologically abnormal Angora goats. Neuronal vacuolation due to intracytoplasmic lipid accumulation has recently been documented in adult raccoons of both sexes from the USA. Isolated clear vacuoles in neurons have also been seen as incidental findings in selected areas of the brain in normal cattle, sheep, pigs, and in dorsal root ganglia of rabbits. Other than in cases of BSE, vacuolation within neuronal perikarya has not been reported in other pathologic conditions of bovines, except in 1 bull with spastic paresis. Disseminated intracytoplasmic neuronal vacuolation in the brain and spinal cord of a neonatal calf is described here.

A male calf was born without difficulty to a Holstein heifer in the dairy herd (175 milking cows) at New Bolton Center (University of Pennsylvania, Kennett Square, PA). The calf was unable to stand unaided but was otherwise normal. When helped to stand, the calf was very unstable on its feet.

Because of the poor prognosis, the calf was euthanized at 6 days of age. Within 1 hour of its death, a complete postmortem examination was done. Gross abnormalities were not seen in the carcass. Representative samples of skin, skeletal muscles (semimembranosus), tongue, rumen, abomasum, intestines, heart, lung, liver, kidney, spleen, urinary bladder, brain, pituitary gland, and the spinal cord were immersed fixed in 10% neutral buffered formalin for histopathology. Immunohistochemistry for protease-resistant prion protein (PrPSc) and for detection of scrapie-associated fibrils (SAF) by electron microscopy was done on the brain. A thin cross-section (approximately 1 mm wide) of formalin-fixed spinal cord was postfixed with 1% osmium tetroxide to prevent loss of material from vacuoles within the neurons during histologic processing. This section was embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE).

Formalin-fixed spinal cord sections with lesions were placed in 2.5% glutaraldehyde in cacodylate buffer, then in 1.0% osmium tetroxide in cacodylate buffer, and then embedded in Epon–araldite. Thick sections (1 μm) were stained with toluidine blue, and selected areas were cut into thin sections, stained with hydrated uranyl acetate and lead citrate, and examined with a transmission electron microscope.

Microscopic lesions were confined to the brain, spinal cord, and skeletal muscles. There was extensive neuronal vacuolation present in the cerebellum, brain stem, and cervical spinal cord (Fig. 1). In the cerebellum, only the roof nuclei were affected (Fig. 1), whereas in the brain stem most of the nuclei, including the dorsal vagus, had variable numbers of vacuolated neurons. When present singly, the vacuoles were quite large (up to 150 μm) and often occupied most of the cell (Fig. 2). When multiple vacuoles were present, the affected neurons appeared foamy (Fig. 1). In the gray matter of the spinal cord, the vacuoles were usually multiple and bilateral but not necessary symmetrical, and many were seen adjacent to normal-appearing neurons. However, in the dorsal root ganglia most of the neurons had a single large vacuole (Fig. 2). At both locations, there was no appreciable increase in glial cells, and no spongiform...
change in white matter or necrotic neurons were observed. Vacuolated neurons were not observed in the ganglion cells of the nonneuronal tissues (tongue, rumen, abomasum, intestines, liver, and urinary bladder). In skeletal muscles, there was mild degeneration and fragmentation of isolated muscle fibers, and moderate numbers of macrophages were present between the fibers.

PrP\textsuperscript{res} and SAF were not detected by immunohistochemistry and electron microscopy, respectively. Ultrastructural examination of the formalin-fixed spinal cord revealed essentially empty spaces that contained scant membranous structures of various sizes (Fig. 3). The empty spaces were not membrane bound.

BSE has not been diagnosed in the USA, and although there is a possibility that prion disease (scrapie) may be transmitted from dam to offspring in sheep (possibly during pregnancy or birth or through suckling),\textsuperscript{14} clinical case of TSE in neonates have not been documented.

Except for 2 reports, a heifer with rabies\textsuperscript{7} and a bull with spastic paresis,\textsuperscript{16} extensive vacuolation of neurons has not been previously reported in cattle without BSE. In this non BSE population only vacuolated neurons were observed, and these neurons were confined to the red and habenular nuclei.\textsuperscript{15} Although vacuolation of neurons has not previously been documented in neonatal calves, similar histopathologic and ultrastructural findings have been reported in young dogs\textsuperscript{12,18} and young goats,\textsuperscript{13} and in both instances, the etiologic diagnosis was not established.

In this neonatal calf, although spongiform change was not seen in the neuropil, severe vacuolation of the neuronal perikarya was widely distributed throughout the central nervous system. The findings of vacuolated neurons by itself would not normally suggest BSE. However, identification of diseases and lesions with morphologic similarities to BSE is important in the differential diagnosis of the disease. Although BSE is not present in the USA, there are other TSEs in US animal populations, such as scrapie in sheep and goats, CWD in cervids, and TME in mink. Therefore, it is essential to rule out any possibility, however remote, of TSE in animals with suspicious clinical signs. In the presently documented case, the ultrastructural studies revealed that the vacuoles were not membrane bound, and the confirmatory diagnostic tests (immunohistochemistry and electron microscopy) were negative for PrP\textsuperscript{res} and SAF, respectively. The conclusion reached was that this calf did not have TSE. However, the final etiologic diagnosis of this condition remains undetermined.

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Figure 3. Electron micrograph of neuronal vacuoles; cervical spinal cord; calf. a. The vacuoles are not membrane bound and contain a few membranous structures of various sizes (arrows). Formalin-fixed tissue, 1,700×. b. Higher magnification of a non-membrane-bound vacuole with a few membranous structures within the clear vacuolated area. 17,000×.

Sources and manufacturers

a. Philips model 410, FEI Co., Hillsboro, OR.

References