Neuronal ceroid-lipofuscinosis in a Holstein steer

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Abstract. A young, partially blind Holstein steer was affected by mild cerebral atrophy. Formalin-fixed cerebral gray matter was diffusely yellow brown. Microscopically, there were eosinophilic, autofluorescent granules primarily in the cytoplasm of cerebral neurons. There was also extensive retinal atrophy with complete loss of the rod and cone layers. Ultrastructural examination of affected cerebral neurons revealed a mixture of granular osmiophilic and lamellar patterns in the cytoplasmic storage bodies. This suggests the existence of neuronal ceroid-lipofuscinosis in the Holstein breed.

Key words: Bovine; ceroid; Holstein; immunohistochemistry; lipofuscin.

Neuronal ceroid-lipofuscinosis (NCL) has been described as a naturally occurring disease in many animal species. The disease is encountered most frequently in dogs, affecting various breeds including the Australian Cattle Dog, Border Collie, Chihuahua, Cocker Spaniel, Corgi, Dachshund, Dalmatian, English Setter, Golden Retriever, Gordon Setter, Japanese Retriever, Labrador Retriever, Miniature Schnauzer, Polish Owczaresz Nizinny, Standard Poodle, Saluki, Tibetan Terrier, and Yugoslavian Shepherd. Neuronal ceroid-lipofuscinoses have also been described in Icelandic/Peruvian Paso horses, Siamese and Domestic cats, ferrets, Nubian goats, Merino, Rambouillet, South Hampshire, and White Swedish Landrace sheep, mnd and nclf mice, a monkey, and Devon and Beefmaster cattle. The term NCL is traditional but not accurate because storage often is not limited to neurons, and the storage bodies in many affected animals (including Devon cattle) have been shown to be composed primarily of an extremely hydrophobic protein—subunit C of mitochondrial adenosine triphosphate synthase (SCMAS)—rather than ceroid or lipofuscin. In some affected animals, especially Miniature Schnauzers and White Swedish Landrace sheep, sphingolipid activator proteins (SAPs) are the primary component of storage bodies.

In humans, NCLs are a common cause of neurodegeneration in children, with some forms additionally or primarily presenting in adults. Human NCLs traditionally have been classified by age of onset combined with ultrastructural phenotype: granular osmiophilic deposits characteristic of SAPs versus the lamellar appearance of SCMAS. However, most human NCLs are now classified on the basis of predicted or defined defects in 7 genes as CLN1 through 8. The CLN1 encodes palmitoyl-protein thioesterase, and the storage material accumulating in affected infants are SAPs, but SCMAS is the main component of the storage material in CLN2-8. The inheritance of many animal and human NCLs has been determined to be recessive, and vision loss has been documented in both human and animal NCLs.

A 15–18-month-old Holstein steer with a history of progressive blindness was presented for slaughter. Grossly, the eyes were unremarkable with the exception of dilated pupils. The animal appeared to have a marked visual deficit, but was afebrile, placid, and well conditioned. Because of the possibility of a central nervous system disorder, the animal was excluded from...
normal slaughter. On gross examination, there were multifocal hepatic abscesses, white foci in the kidney, and cerebral folia appeared shrunken. Cerebrum, liver, kidney, spleen, eye, lung, and renal and hepatic lymph nodes were placed in 10% neutral-buffered formalin. There was a light yellow-brown discoloration of the gray matter of the formalin-fixed cerebrum.

Formalin-fixed tissues were routinely processed, embedded in paraffin, 4 μm sections were placed on glass slides, stained with hematoxylin and eosin (HE), and examined by light and fluorescent microscopy. Some replicate sections were stained with periodic acid–Schiff (PAS), Sudan Black, or Luxol-Fast Blue (LFB). The HE-stained and unstained sections were examined by reflected light fluorescent microscopy using a combination of exciter and barrier filters that resulted in bright lines at 334–365 nm. Some sections of cerebrum and retina were immunostained for glial fibrillary acidic protein (GFAP) and synaptophysin using a supersensitive streptavidin–biotin–alkaline phosphatase kit and antigen retrieval (Antigen Retrieval Citra Solution), as described previously. Sections of unaffected bovine cerebrum and retina were used for positive and negative controls for immunostaining with the specific primary antibody omitted in negative controls. For ultrastructural examination, 1-mm cubes of affected cerebrum were removed from paraffin blocks, cleared twice by immersion in xylene, rehydrated, and further fixed in a solution of 2% glutaraldehyde, 2% paraformaldehyde, and 0.2% picric acid in a cacodylate buffer (pH 7.3). Tissues were postfixed in osmium, processed, and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with transmission electron microscope.

In HE-stained sections, the cytoplasm of cerebral neurons contained nearly colorless to moderately eosinophilic, refractile inclusion bodies that ranged from 1 to 8 μm in size (Fig. 1). Individual neurons often contained 3–10 granules, but in some neurons there appeared by light microscopy to be a single storage body, whereas other neurons contained numerous, well-dispersed granules. Neuronal inclusions were often irregularly round to ovoid, perinuclear, and tended to accumulate in the cytoplasm, along one margin of the nucleus. Some larger granules were composed of aggregates of numerous smaller bodies. The granules were light pink with the PAS stain, moderately dark blue with LFB, and dark gray to black with Sudan Black. Under fluorescent light, granules in HE sections were brilliantly autofluorescent (Fig. 2), and granules in unstained sections exhibited a bright-yellow autofluorescence. Larger granules were often composed of aggregates of small granules. There was limited accumulation of storage material in the cytoplasm of some neuroglial cells, occasional macrophages, and rare cerebral endothelial cells and evidence of neuronal necrosis with accompanying neuronophagia and astrocytosis. Glial fibrillary acidic protein immunostaining was more intense than control tissues throughout the cerebral gray and white matter without a distinct laminar pattern but was most intense in the deep cortical gray matter. There was a laminar band of relatively less-intense synaptophysin immunostaining in the superficial cerebral gray matter.

In the retina, there was complete loss of the layer of rods and cones and the outer nuclear layer. The inner nuclear layer (bipolar cell layer) was only mildly depleted, but the ganglion cell layer was markedly depleted, often with only isolated, swollen ganglion cells remaining. There was moderate thinning of the inner plexiform (synaptic) layer and extensive loss of the outer plexiform (synaptic) layer. A few macrophages near the remnants of the outer limiting membrane contained nearly colorless granular cytoplasmic inclusions that were brightly autofluorescent; rare similar mac-
Rrophages were present in the depleted inner nuclear layer. By fluorescence microscopy, bipolar nerve cells in the inner nuclear layer often contained small, yellow cytoplasmic granules, and ganglion cells often contained similar but finer cytoplasmic granular inclusions. In the affected retina, GFAP staining was generally more intense than the normal retina, especially in the nerve fiber and ganglion cell layers and in the atrophic outer retina. Synaptophysin immunostaining of the affected retina showed normal staining intensity in the inner plexiform layer, but this layer was moderately thinned. Immunostaining of the nerve fiber and outer plexiform layers was markedly decreased, and the layer of staining normally present near the outer limiting membrane was absent.

There was a mild chronic interstitial nephritis with radial streaks of cortical interstitial fibrosis accompanied by accumulations of lymphocytes and plasma cells. Rarely, the epithelium of some cortical tubules contained small, often single, nearly colorless inclusions that were autofluorescent by fluorescence microscopy. There was also a moderate to marked chronic cholangiohepatitis, and some macrophages in fibrotic portal triads contained autofluorescent cytoplasmic inclusions, as did rare hepatic Kupffer cells.

By electron microscopy, neuronal storage bodies often appeared as discrete, partially membrane-bound, electron-dense (osmiophilic) cytoplasmic bodies (Fig. 3), which variably incorporated multilaminar arrays (Fig. 4). Smaller storage bodies often were surrounded by distinct electron-dense lamina, but larger storage bodies, although well defined, were often incompletely membrane bound. There were abundant mitochondria in the adjacent cytoplasm; some appeared to have distorted or thickened cristae.

Retinal changes in this young steer are similar to those described in the Devon breed, with marked loss of the layer of rod and cones and associated outer nuclear layer and less extensive loss of neurons in the ganglion cell layer. The marked loss of retinal synaptophysin immunostaining, especially the loss of the outer plexiform layer, likely represents the loss of synapses associated with rod and cone cells because synaptophysin is a presynaptic protein in chemical synapses.

By light microscopy, most storage bodies in cerebral neurons appeared to be more coarsely granular than those described previously in cattle. There appeared to be a loss of specific populations of neurons in the cerebrum, although the distinctive laminar necrosis described in Devon cattle was not demonstrated. Some researchers have suggested apoptosis as the cause of this targeted neuron loss, whereas others have focused on metabolically active, mitochondrial-rich neurons suggested to be damaged because of mitochondrial dysfunction and chronic neuronal excitotoxicity. The multilaminar ultrastructural characteristics of sections of the neuronal storage bodies in this young steer are most consistent with some storage of SCMAS. This extremely hydrophobic mitochondrial protein often forms insoluble complexes with lipids, and its accumulation in many NCLs has led to the theory that there may be a defect in its transport or degradation. A yeast model in which a homologous gene for CLN3 was disrupted suggested that defects in the control of endosomal/lysosomal pH may play a role in this accumulation of SCMAS. This inference was reinforced by the production of an NCL-like phenotype (including retinal atrophy) in a mouse model by the disruption of a gene coding for a chloride channel because the proposed pathogenesis also involved elevated endosomal pH.

We have described a partially blind Holstein steer affected by neuronal ceroid-lipofuscinosis that exhibited retinal and cerebral atrophy. The emergence of
this lipoprotein proteinosis in a breed, which depends to a great extent on artificial insemination for calf production, implies there is a possibility that the presumed genetic defect could be more rapidly disseminated within this breed and additional affected animals may be identified.

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Sources and manufacturers
a. BioGenex Laboratories, San Ramon, CA.

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