"Shaker" Calf Syndrome: A Newly Recognized Inherited Neurodegenerative Disorder of Horned Hereford Calves


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Abstract. The clinical and pathological features of a newly recognized, inherited neurodegenerative disorder in horned Hereford calves are described. The disorder is expressed in newborns by tremulous shaking of the head, body, and tail, difficulty in rising, a wobbly spastic gait, and aphonia. Transient improvement is followed by deterioration and progressive spastic paraplegia. Generalized tremors can be induced easily by a variety of stimuli, and spinal reflexes may be exaggerated or depressed. The major pathological finding is an excessive accumulation of neurofilaments within neurons of the central, peripheral, and autonomic nervous systems. The involvement of multiple systems of neurons and the similarity with some forms of human motor neuron disease and spinocerebellar degeneration suggest that this unique bovine disease may serve as a suitable animal model for these human neurodegenerative disorders.

Several inherited neurodegenerative diseases of cattle have been described in various breeds. Some diseases involve accumulations of storage products, as in mannosidosis in purebred Angus[33,35] GM-1 gangliosidosis in inbred Friesians,[17,19] and neuronal lipodystrophy in Beefmasters.[46] Other diseases are distinguished by widespread focal swelling of axons, such as hereditary neuraxial edema[11] and some diseases include neurological signs such as epilepsy and tremors, without any accompanying pathological findings. Examples in this last group are: inherited epilepsy,[1,32,45] spastic syndrome,[2] and "daddlers."[26]

This report describes one of several calves born with severe neurological signs and a generalized nervous disorder of the nervous system characterized by excessive accumulation of neurofilaments within neurons.

Clinical History

The syndrome was first noted in 1980 in a herd of horned Hereford cattle consisting of 75 registered purebreds and 120 commercial cows, in southern Alberta, Canada. The female calf described in this report was one of six affected during 1980 and 1981 from this herd. All diseased calves were born normally but were unable to stand without assistance, and several hours later developed fine generalized tremors that were pronounced in the neck and hindlimbs. Stimulation, by handling or excessive spontaneous activity, increased the amplitude and frequency of these fine tremors. Tremors were accompanied by an exaggeration of bodily movements with progressive loss of muscular strength due to fatigue. A few calves either died through inanition or developed pneumonia—necessitating euthanasia. However, the calf in this report improved, and by day 5 of life was able to nurse with assistance and appeared strong. Remission was only temporary; at two weeks postpartum the calf began to deteriorate. By three months of age, the calf displayed spastic paraparesis, had difficulty in rising, and at times could not maintain sternal recumbency. Its gait, especially in the hindlimbs, was stiff, wobbly, and incoordinate, and the animal fatigued with continued exercise. Although there was no gross wasting of muscles, the extensor groups appeared weaker than the flexor groups. From birth the heifer was aphonic, and in this terminal stage was unable to coordinate tongue movements, which resulted in tremulous attempts to lick the nose.

The pupillary light reflex was sluggish, and reflexes of the left patellar, right gastrocnemius, and right triceps deep tendon were depressed; reflexes of the right patellar and left triceps deep tendon were increased, and clasp knife reflexes were exaggerated. Hyperesthesia to tactile stimulation was demonstrated easily in association with aggravation of the generalized tremors. Despite these neurological difficulties the calf remained bright and alert.

Materials and Methods

The affected calf and a control calf of similar age were killed by intravenous injection and immediately necropsied.
Tissue sections of the brain, spinal cord, eye, sympathetic autonomic ganglion, lung, heart, liver, spleen, small and large bowel, thymus, mesenteric lymph nodes, gland, adrenal glands and thyroid were fixed in 10% neutral buffered formalin and embedded in paraffin. However, no sections of skeletal muscle or peripheral nerve were taken. Histological sections of neural tissues were stained with hematoxylin and eosin (HE), and luxol fast blue-hematoxylin and eosin. In addition, period acid-Schiff (PAS), Nissl cresyl violet, Bodian silver for axons and neurons, and Holzer stain for reactive astrocytes were done on sections of spinal cord from the affected calf.

Selected samples of brain, spinal cord, and sympathetic autonomic ganglion were taken at the time of necropsy and fixed in chilled 4% glutaraldehyde in 0.1 M cacodylate buffer. The tissue was postfixed in 1% osmium tetroxide, processed for electron microscopy, and embedded in epon. Two μm sections stained with toluidine blue were used for screening. Ultrathin sections were cut on an ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

Serum, urine, thymus, spleen, liver, heart, and kidney were collected from this afflicted calf, the dam of the calf, and an age-matched control calf for biochemical analysis. Whole blood and kidney were collected for karyotyping.

**Results**

The salient pathological findings in the affected calf were an ongoing neurofilamentous neuronal degeneration of multiple cell groups within the central nervous system and of ganglion cells within the peripheral and autonomic nervous systems. The spinal cord was most severely affected; all segments had diffuse involvement of the somatic motor neurons in the ventral gray horns and major sensory relay nuclear groups in the substantia gelatinosa. The thoracic nucleus (Clarke's column) and the sympathetic autonomic motor neurons in the intermediolateral nucleus also were afflicted. The neuronal degeneration was characterized by swelling of the perikaryon and distension of the neuritic processes by an opalescent, faintly fibrillar, amphophilic material, which appeared to be arranged in whorls as outlined by entrapped, coarsely clumped Nissl substance (fig. 1). This material did not stain with luxol fast blue or by the PAS technique. Bodian silver preparations revealed fine argentophilic fibrils which coursed through the amphophilic material and extended into the neuritic process, but did not form fibrillary tangles. Because of the marked swelling of the neuronal cell body, the nucleus was eccentrically displaced but there was no vacuolar degeneration. Although neuronophagia was infrequent, there were occasional glial nodules and empty cell nests present mainly in the lateral half of the ventral gray horn. A mild reactive gliosis was evident in the Holzer preparation.

Concurrent wallerian-like axonal degeneration was demonstrated in the ventral spinal roots and intramed-

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**Fig. 1:** Motor neurons in ventral gray horn of cervical spinal cord. *a.* Control calf. *b.* Affected calf; marked swelling of neuronal cell bodies and enlargement of neuritic processes (arrows). Occasional empty cell nests (*). HE. Bar = 100 μm.
ullary rootlets as well as in the spinal white matter. In the spinal roots, this process was represented by focal eosinophilic axonal swellings (spheroids) and sporadic, linear rows of digestion chambers of myelin balls within autophagocytic Schwann cells. Similarly, in the white matter there were small numbers of large axonal spheroids undergoing phagocytosis by macrophages to form empty fiber tracts. This mild fiber degeneration occurred within a ventromedial sector in the ventral columns, the central region of the lateral columns, and with less frequency, throughout the dorsal columns. In the few dorsal spinal roots examined, rare axonal spheroids were present.

Similar neuronal degeneration occurred in other regions of the neuraxis, but fewer cell groups were involved and the extent of the alterations was less. In the brainstem, as in the spinal cord, there was marked involvement of all major motor nuclei as well as the large multipolar neurons of the reticular formation. Involvement of other nuclear groups appeared to be minor with only occasional swollen neurons present in the accessory cuneate nucleus, the principal and spinal trigeminal sensory nuclei, and the pontine nucleus. Cerebellar involvement was represented by uniform perikaryal swelling of the Purkinje cells, while in the cerebrum early degenerative changes were seen in sporadic neurons of the lateral geniculate body and in the fifth cell layer of the frontal cortex. These early changes consisted of peripheral striate clearing of the perikaryal cytoplasm, dispersion of Nissl substance, eccentric displacement of the nucleus, and enlargement of neuronal processes. In the eye, the retinal ganglionic cell layer was involved. In all these sites, however, there was no conspicuous loss of neurons or ongoing degeneration of fiber tracts.

Involvement of the peripheral and autonomic nervous system was represented by the presence of a few ganglion cells that displayed perikaryal degenerative swelling within the trigeminal ganglion, cervical dorsal root ganglia, and sympathetic autonomic ganglion. The noted paucity of axonal spheroids in the dorsal spinal roots was approximately proportionate to the small numbers of degenerating neurons in the dorsal root ganglia. Additional changes within the peripheral and autonomic ganglia included proliferation of satellite cells, neuron neuronophagia, and numerous residual nodules of Nageotte. Further involvement of the autonomic nervous system was manifested by swelling of ganglionic cells in Auerbach’s myenteric plexus of the small bowel. No muscle or nerve was studied to ascertain distribution of the disease in the distal peripheral nervous system.

Electron microscopic examination of the spinal ventral gray horns revealed that the motor neuronal cell bodies were massively distended by densely packed, interlacing, whorled arrays of neurofilaments that surrounded and displaced the nucleus and extended into the dendritic processes (fig. 2). These neurofilaments averaged 12 nm in diameter, possessed short side arms, pursued a straight course, were arranged in bundles, and did not form tangles or twisted, paired helical arrays. The remaining neuronal organelles were segregated into isolated clumps and were dispersed randomly throughout the perikaryon among the whorled bundles of neurofilaments. No microtubules were present. The myelinated axons within the neuropil also had dense packing with neurofilaments. Similar excessive accumulations of neurofilaments were found in neurons in the trigeminal, dorsal root, and sympathetic autonomic ganglia.

The possibility of a mucopolysaccharidosis was assessed by quantitative uronic acid determination of cetyltrimethylammonium bromide precipitated urinary glycosaminoglycans. Urinary uronic acid was elevated in the affected calf (57 μg/ml) compared to a range of < 2 to 4.8 μg/ml for control calves. Electrophoresis of the precipitated glycosaminoglycans revealed the presence of only chondroitin 4 and 6 sulfates which are normal components of urine.

Several tissue and serum activities were assayed using fluorogenic substrates according to published procedures. These were α-mannosidase, α-fucosidase, β-galactosidase, β-glucuronidase, N-acetylhexosaminidase, and arylsulfatase. There were no apparent differences for these activities between the affected and control calf in either thymus, spleen, liver, or kidney. Serum hexosaminidase, however, was moderately elevated in the affected calf, 169 nmole/hr/mg protein as compared to 78 ± 13 nmole/hr/mg protein for control calves. Cytogenetic studies did not reveal any chromosomal abnormalities.

Figure 3a illustrates the probable genealogy of the affected calves in the commercial herd, in which the records are not reliable. As noted in the legend, the sire of these calves cannot be determined with certainty. This herd of cows was with eight bulls in a large pasture in which DI was one of the eight bulls, and six of the remaining seven bulls were paternal half siblings to DI.

Figure 3b shows the genealogy of the affected calves in the registered purebred herd that consisted of 75 animals in the 1979 breeding season and 81 animals in the 1980 breeding season. The purebred cows were artificially inseminated with semen from bull DI and then placed with the commercial herd of 120 cows with...
eight bulls—including bull DI and seven others, of which six were half siblings to DI. The 1979 breeding produced two affected calves. In 1980, bull DI was bred to DII and DIII; both cows had been sired by AI, and had produced affected calves in 1980.

In 1981 bull DI was bred to six cows which had previously produced affected or suspected affected calves. Three affected calves were born to these matings. The mode of inheritance and the degree of penetrance of this congenital defect are difficult to establish without further breeding trials. This condition was likely produced by a 12.5% inbreeding factor that resulted in a large degree of genetic homozygosity.

**Discussion**

The main clinical features of the disorder affecting the heifer in this report and other involved calves were the onset of generalized tremors, wobbly spastic gait with difficulty in standing, and aphony after birth. The course of the disease was variable with early death through inanition, or transient recovery followed by progressive deterioration over several weeks—resulting from spastic paraparesis associated with stimulus-sensitive tremors. Pedigree analysis suggested this to be an inherited disorder. The key pathological finding was an excessive accumulation of neurofilaments within neurons in the central nervous system, ganglionic cells in the retina, and peripheral and autonomic nervous systems. Involvement of the major motor and sensory neuronal groups in the spinal cord and brainstem was prominent and was accompanied by minor cell loss and wallerian-like axonal degeneration. Although there was some correlation between the neurological signs and the lesions, the widespread distribution of neuronal degeneration thwarted any precise clinico-pathological correlation.

Inherited degenerative disorders in cattle can be differentiated in this calf on both clinical and pathological grounds. Mannosidosis, GM-1 gangliosidosis, and other lysosomal storage diseases involve storage of metabolic products that cannot be processed due to the lack of production of an enzyme as a result of presence of a faulty gene. In these diseases, there is PAS-positive material found within saccular dilatations of the Golgi apparatus in neurons, astrocytes, microglia, pericytes of blood vessels and macrophages. Spastic paresis and the spastic syndrome, lethal spasms in Herefords,
and "Doddler's Syndrome" have also been described but are pathologically and clinically different from the "shaker" calf. Hereditary neuraxial edema in neonatal polled Herefords is represented by microscopic spongy vacuolar appearance of the central nervous tissue along the long axis of myelinated fibers in the white and gray matter, whereas horned Hereford calves with congenital brain edema have severe myelin breakdown with myelin debris that contains macrophages and swollen hydropic astrocytes in the central cortex.

Occasional reports recently have described spontaneous motor neuron disease in different species of animals in conjunction with neurofibrillary neuronal degeneration. The most extensively studied entity is the hereditary canine spinal muscular atrophy of Brittany spaniels. In this disease, there is a generalized muscle weakness which is most severe in the proximal axial and appendicular muscles. Atrophy and weakness of bulbar muscles develop later with the terminal state being a flaccid tetraplegia. Spinal and postural reflexes are depressed; sensory functions appear intact. The age of onset and progression of the disease varies and three distinct forms have been identified: early onset or accelerated, intermediate onset, and late onset. The major pathological finding consists of a massive aggregation of neurofilaments within the perikarya, dendrites, and proximal axonal segments of the motor neurons in the spinal ventral gray horns and the hypoglossal and trigeminal motor nuclei. Unlike the "shaker" calf syndrome, there is no involvement of the oculomotor nuclei, sensory nuclei in the spinal cord and brainstem, or neuronal populations in the cerebellum, cerebrum and peripheral and autonomic nervous system, nor is there spinal tract degeneration.

The sporadic occurrence of a disorder with similar clinical and pathological features to hereditary canine spinal muscular atrophy has also been described in a domestic cat. More like the "shaker" calf syndrome is a stereotyped form of lower motor neuron disease in zebra foals, crossbred rabbits, and Yorkshire pigs. In each species of animal this disorder has a juvenile onset, is characterized clinically by hindlimb ataxia and weakness rapidly progressing to tetraplegia after approximately one month's duration, and appears to be
Inherited. The lesions are generally the same in each species, and consist of neuronal neurofilibrillary degeneration of the spinal ventral gray horns, cranial nerve nuclei, reticular formation, and red nucleus. Wallerian-like axonal degeneration has been observed in the ventral spinal roots, and ventromedial and lateral columns of the spinal cord. However, no abnormalities have been reported in the cerebellum, cerebrum, retina, dorsal root ganglion cells, or autonomic nervous system.

The closest description of a neurodegenerative disease resembling the “shaker” calf syndrome is a report of a blind, tetraplegic 12-week-old Collie pup. In this condition, accumulations of 12 nm neurofilaments are present in neurons and proximal axons within the ventral gray horn of the first cervical spinal segment, many of the motor and sensory nuclei in the brainstem, Purkinje cells in the cerebellum, occasional pyramidal neurons in the fifth layer of the cerebral cortex, and the retinal ganglion cells. Unfortunately, because the neuropathological examination had been limited to the brain, the extent of involvement of other components of the nervous system in this dog is unknown. In comparison with these other motor neuron diseases with neurofibrillary degeneration, the uniqueness of the “shaker” calf syndrome is that all three components of the nervous system are involved, with widespread distribution in the central nervous system.

The mechanism for the neurofibrillary lesions in the “shaker” calf syndrome is speculative. From the available knowledge concerning the function, synthesis, assembly and catabolism of neurofilaments within neurons it is known that the neurofilament is involved in the slow component of axonal transport and is composed of three major protein moieties having molecular weights of approximately 68,000, 150,000 and 200,000 daltons. The ultrastructural features of the neurofilament as a straight filamentous profile with branching 2.5 nm side arms, an average diameter of 10 nm, and an indefinite length place it in the category of cytoskeletal intermediate filaments, and suggest an additional role in the maintenance of the shape of the neuron and neuritic processes. In the “shaker” calf syndrome, an underlying defect in neurofilibrillary metabolism is implied by a genetic basis as indicated from the pedigree analysis, the neuronal accumulation of neurofilaments, and the preferential selection for nuclear groups composed of large metabolically active neurons. Further suggestive of a metabolic dysfunction, possibly related to lysosomal involvement, is the observation of elevated uronic acid and serum hexosaminidase levels in the affected calf, although these findings may be nonspecific. Similar neurofibrillary degeneration can be induced experimentally in the same neuronal populations as in the bovine neuroaxonal disorder by a variety of intoxicants: aluminum, vincristine and choline. For many of these experimental degenerations a defect in axonal transport has been hypothesized or demonstrated to be related to the accumulation of filaments, and the observation of an impairment in the slow component of axonal transport in β-β' iminodiopropionitrile intoxication has been extended recently to hereditary canine spinal muscular atrophy. Therefore, it is possible that a related abnormality of axonal transport is present in the “shaker” calf syndrome although errors in the synthesis, assembly or catabolism of the triplet neurofilamentous proteins cannot be excluded at this time.

The “shaker” calf syndrome is potentially useful as an animal model for two major types of human neurological disorders: motor neuron disease and the spinocerebellar degenerations. Encompassed under the heading of motor neuron disease are several different entities which have in common the loss of motor neurons in the spinal cord and bulbar brainstem. Accumulations of neurofilaments within the perikarya and proximal axons of the degenerating motor neurons, similar to that noted in this bovine disorder, have been described in the sporadic adult form of motor neuron disease, amyotrophic lateral sclerosis, or of juvenile onset, and Werdnig-Hoffmann disease, a hereditary infantile form. Spinocerebellar degenerations are ill-defined groups of disorders of which Friedreich's ataxia is the best known. The lesions are characterized by neuronal loss in a pattern of involvement similar to that noted in this calf disease: dorsal root ganglia, Clarke's column, brainstem and cerebellar nuclei, and retina. There is also secondary tract degeneration in the spinal cord. A recent report further supports a possible parallel between Friedreich's ataxia and the “shaker” calf syndrome; it describes axonal accumulations of neurofilaments in a biopsy of dorsal root ganglia.

Additional studies are being done to further delineate the clinical and pathological features of the “shaker” calf syndrome. The identification of heterozygotic dams or sires may aid in control of this disease through the bovine genetic pool—reducing potential economic loss which results from the production of nonviable calves. Furthermore, by exploring this disease further, an understanding of the pathogenesis of some human neurological disorders, which could not be examined previously in detail, may be obtained.
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