Leukomyelopathy in Ataxic Calves

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ABSTRACT. Seventy-three fattening calves ranging from 6 to 15 months old on a farm were affected by ataxia over a period of four months. About 10 ataxic animals recovered after the administration of various kinds of vitamins and needle puncture therapy. The principal lesions in 14 ataxic animals examined were characterized by bilateral vacuolation with axonal degeneration confined to the spinal white matter. Ultrastructurally, the vacuoles were present in the periaxonal areas and bounded by dissociated myelin lamellae. No pathogen was isolated from the visceral organs of the animals. The serum copper remained within the normal range. Possible etiological factors remain unknown in the ataxic disease.—**KEY WORDS:** ataxia, fattening calf, leukomyelopathy.

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Status spongiosus or spongy degeneration refers to lesions characterized by striking vacuolation of the central nervous system (CNS) resulting in a sieve-like appearance [13]. Electron microscopical examinations reveal that intracellular, extracellular, and intramyelinic accumulation of fluid as well as artificial dissolution of myelin may account for the spongiform changes based on light microscopical observations [2].

Intramyelinic edema is observed as vacuoles in the white matter of the CNS by light microscopy, and as intramyelinic vacuoles that developed after splitting of the intraperiod line of the myelin sheath by electron microscopy [2]. Intramyelinic edema was produced experimentally in the CNS of laboratory animals by using a number of toxic substances such as triethyltin [8], isonicotinic acid hydrazide [2] and cuprizone (biscyclohexanone oxalyldihydrazone) [21]. Similar changes have been reported in hepatic encephalopathy with hyperammonemia [3, 9–11] and in hereditary or possibly hereditary diseases of newborn or young animals [4–6, 14, 16, 17, 19, 26] and human infants [1], but there are also several animal diseases [20, 25] with similar changes in the CNS whose causal agents are still unknown.

This study describes the pathological findings of ataxic calves with vacuolation confined to the white matter of the spinal cord, and comparison between the present findings and previous reports of ruminants with similar spinal lesions [12, 20, 22, 24].

On a farm located in the south of Iwate Prefecture, Japan, about 700 head of castrated male Holstein-Friesian cattle, which were from many farms in the prefecture, had been raised for fattening in 7 barns consisting of a total of about 30 pens since one or two weeks of age. In the period of 4 months from December, 1991 to the following March, 73 calves were affected by non-fatal ataxia. All affected animals had grown normally to the age of between 6 and 15 months when their clinical abnormalities were observed. The occurrences were found in 15 pens in 5 barns, and 32 (44%) of the 73 affected cattle showed signs of ataxia in 2 pens in two barns.

Most of the affected animals showed signs of ataxia on either the thoracic or lumbar limbs bilaterally. The

thoracic limbs were somewhat more affected than the lumbar limbs. Several of the animals affected more severely had showed signs of ataxia on both thoracic and lumbar limbs since the onset of the disease. Clinical abnormalities in the animals were indicated in the affected limbs by a stiff gait, followed by inability to stand within the following half day. Their attempts to rise were unsuccessful though repeated, since they kept their carpal or tarsal articulations on the floor with flexuous limb toes backward, bilaterally. These postures suggested distal paralysis of the carpal or tarsal articulations. The animals were afebrile, alert, responsive to visual and auditory stimuli, and able to drink or graze successfully. Other signs included mildly excessive salivation and a slight decrease in rumenal movement.

About 10 of the affected animals recovered and could rise without assistance within a week after repeated treatments with electrolytes and various kinds of vitamins including B1, B2, B6, B12, and C and puncture stimulation by medical needles on the peripheral nerves distributed to the affected limbs. The clinical improvement was maintained over the next several months to a year until the animals were slaughtered after sufficient fattening. The other animals did not respond to the treatments, and were slaughtered or euthanased for necropsy 1 to 3 weeks after the onset of the disease. These clinical signs did not progress within 3 weeks of the observation period. The calves had been fed the same kinds of feeds as the ones on the other farms where no ataxic disease occurred. No parasiticides were administered to any animals in the farm.

In the same period, 32 calves which had been raised with the ataxic ones in the barns were found dead though no signs of the disease had been observed previously. Clostridial vaccines including *Cl. septicum*, *Cl. chauvoei* and *Cl. perfringens* were administered to all animals aged more than 4 months in late March, 1992. The occurrence of both fatal and ataxic conditions has disappeared since April.

Fourteen ataxic and 4 fatally affected calves were submitted for macroscopical and bacteriological examinations. Histological and electron microscopical examina-

Table 1. Distribution of vacuolar lesions in the spinal white matter and affected limbs

Case No.	Cervical				Thoracic				Lumbar				Affected limbs
	DF ^{a)}		LF ^{b)} &VF ^{c)}		DF		LF&VF		DF		LF&VF		
	DP ^{d)}	SP ^{e)}	DP	SP	DP	SP	DP	SP	DP	SP	DP	SP	
1	-	-	-	-	-	-	-	-	-	-	+	-	lumbar
2	-	-	-	-	-	-	+	-	-	-	-	-	thoracic
3	-	-	-	-	-	-	++	-	+	-	++	-	lumbar
4	++	+	+	-	++	-	+++	+	+++	++	+++	+	both
5	-	-	-	-	-	-	+	-	-	-	-	-	thoracic
6	-	-	+	-	-	-	+++	+	+	-	+++	+	both
7	-	-	-	-	-	-	+	-	-	-	+	-	thoracic
8	-	-	-	-	-	-	+	-	-	-	+	-	thoracic
9	-	-	-	-	-	-	+	-	+	-	++	-	lumbar
10	-	-	-	-	-	-	+	-	+	-	+	-	lumbar
11	-	-	-	-	++	-	++	-	+	-	+	-	thoracic
12	-	-	-	-	-	-	+	-	-	-	+	-	lumbar
13	-	-	-	-	+	-	++	-	-	-	+	-	thoracic
14	-	-	-	-	++	-	+	-	++	+	+++	-	lumbar

a) DF: dorsal funiculus, b) LF: lateral funiculus, c) VF: ventral funiculus, d) DP: deeper portion, e) SP: superficial portion. +: mild, ++: moderate, +++: severe lesions.

tions were performed on 14 and 4 ataxic calves, respectively. Copper was analyzed on serum samples from 14 ataxic cases. Macroscopically, no lesions were found in the CNS, peripheral nerves, skeletal muscles or the thoracic or abdominal viscera in any of the ataxic animals. On the other hand, all the fatally affected animals had marked hemorrhage and congestion throughout the body.

Microscopically, the principal lesion in the ataxic animals was a myelopathy with vacuolation confined to the white matter. The lesional distribution was bilateral. The animals with anterior or posterior limb ataxia had predominant changes in the thoracic or lumbar segments, respectively. The deeper portions of the lateral and ventral funiculi were more frequently and severely affected than other portions of the funiculi. Though the deeper portions of the dorsal funiculi were also involved, the frequency and severity of the lesions were generally mild. The lesions in the superficial portions of all funiculi were occasionally present and mildly affected (Table 1).

The affected white matter in transverse sections had a spongy appearance due to numerous vacuoles (Fig. 1). Most vacuoles were empty and some contained swollen axons. There were macrophages in a few vacuoles. The lesions in longitudinal sections stained with Bodian's silver impregnation (Bodian) were characterized by numerous dilated periaxonal spaces with degeneration or loss of axons. The dilated spaces were partitioned by delicate septa stainable with Luxol fast blue (LFB), and contained swollen axons. There was fragmentation or loss of axons in the severely dilated spaces (Fig. 2). Segregated "digestion chambers" containing macrophages and cellular debris were occasionally found. No distinct deficiency of myelin was present in any of the sections stained with LFB. There was no appreciable inflammatory or vascular response to the vacuolation, though mild hypertrophy of astrocyte nuclei was seen in the lesions.

For electron microscopical observation, 6.25% glutaraldehyde-prefixed or formalin-fixed spinal cords from one (Case No. 12) or 3 (Case Nos. 4, 6, 14) ataxia cases were postfixed in 1% osmium tetroxide. They were then processed routinely for embedding in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate. The vacuoles were present in the periaxonal areas with various degrees of dilation, and bounded by dissociated myelin lamellae in the spinal white matter. Inner myelin lamellae separated from the rest of the sheath and formed some vacuoles in the mildly dilated periaxonal areas (Fig. 3). Swollen axons containing numerous neurofilaments were often found and macrophages phagocytosing myelin occasionally observed in the moderately or more dilated periaxonal areas (Fig. 4).

Gray matter involvement was limited to the sections with severe white matter lesions and consisted of a few vacuoles in myelinated fibers passing through the gray matter. The spinal nerve rootlets and ganglions were intact.

There were no significant lesions in the brain except for single or a few chromatolytic neurons in the red nucleus of the midbrain. The optical retinae and nerves, peripheral nerves such as axillary and sciatic, and skeletal muscles such as supraspinatus, carpo-ulnar extensor, semimembranosus and sura-tricipital, were also intact. Lesions in the other organs or tissues were sporadic vascular changes consisting of hyaline degeneration of media and swollen endothelial cells in the walls of the digestive tract, gallbladder, and urinary bladder, and mild cloudy swelling of hepatic cells.

The visceral organs, brain, spinal cord, and duodenal and jejunal contents were used for aerobic culture on tryptic soy agar supplemented with 5% sheep blood, or for anaerobic culture on GAM agar with a gas generating kit (Oxoid Limited, England) at 37°C for 48 hr. Isolates were

Fig. 1. Marked vacuolation of the white matter in the transverse section of the 2nd lumbar segment of case No. 4. Macrophages (arrows) are found in several vacuoles. HE stain. $\times 143$.

Fig. 2. Dilated periaxonal spaces with swollen or fragmented axons are observed in the longitudinal section of the white matter from the 3rd lumbar segment of case No. 4. Dilated periaxonal spaces are partitioned by delicate septa stainable with LFB. LFB-Bodian stain. $\times 320$.

Fig. 3. Ultra-thin section of the white matter from the 2nd lumbar segment of case No. 12. Inner myelin lamellae (arrow) separate from the rest of the sheath and form some vacuoles in the mildly dilated periaxonal areas. $\times 14,500$.

Fig. 4. Ultra-thin section of the white matter from the 2nd lumbar segment of case No. 4. A swollen axon and macrophage (M) phagocytosed myelin are found in the dilated periaxonal area. $\times 3,500$.

identified according to Bergey's manual. For typing of isolated *Clostridium perfringens*, 5 kinds of type-specific antisera were used. No bacteria were isolated from any materials from any of the ataxic animals. On the other hand, *Cl. sordelli* or *Cl. perfringens* type A were obtained from the parenchymatous organs or the duodenal and jejunal contents of the fatally affected animals. Serum copper content was examined by atomic absorption spectrometry (SAS 760, Seiko Instruments Inc., Japan). The values ranged from 0.73 to 1.64 $\mu\text{g/ml}$ in all the animals examined. The mean value was 1.037 $\mu\text{g/ml}$ with a standard deviation of 0.289.

The clinical signs of the present ataxic disease were not proven to be progressive, and about 10 affected animals showed signs of recovery from the clinical abnormalities. The finding of many diseased animals within a limited period suggests the involvement of infectious or toxic agents as possible etiological factors, but there was no clear evidence to suggest these agents. The fact that the affected animals were from many farms without any ataxic disease, seems to exclude the possibility of a hereditary etiology.

The lesions observed in the present ataxic disease were characterized by dilated periaxonal spaces with degeneration or loss of axons confined to the white matter of the spinal cord. The lesions were bilateral and predominant in the deeper portions of the lateral and ventral funiculi which are rich in association fibers interconnecting the spinal segments. The bilateral lesions suggest a metabolic disorder for the pathogenesis [23]. The predominant distribution of the lesions in the deeper portions suggests that association fibers are affected more frequently than afferent or efferent ones. Ultrastructural examination revealed that vacuoles were present in the periaxonal areas and bounded by dissociated myelin lamellae.

There are a few reports [12, 20, 22, 24] on myelopathy of ruminants with lesional distribution confined to or predominant in the white matter. They include progressive spinal myelinopathy in beef cattle [20]. This disease was different in some respects from the present ataxic disease. The former disease was progressive in the clinical signs and lesional severity, and showed predominant lesions in the superficial white matter of the spinal cord with a distinct deficiency of myelin and dilated myelin sheaths [20]. Ataxia associated with copper deficiency in red deer [22, 24] and sheep [12] is known as a degenerative spinal cord condition confined to the white matter. The lesions in deer and sheep were, however, characterized by demyelination in the lateral and ventral funiculi [12, 22, 24]. The serum copper in the present ataxic animals remained within the normal range. It therefore seems that there is little relation between the disease and copper deficiency.

Spongiform myelinopathies of animals in which the dominant pathological feature is dramatic vacuolation of myelin, may fall into two broad groups: those which are idiopathic, and those which have a defined metabolic or toxic basis [15]. Several conditions involving newborn

Hereford calves [4, 14], which are classified into the former group, were clinicopathologically characterized by inherited congenital myoclonus and well-developed vacuolation in the cerebrum, brain stem, and cerebellum. The toxic or metabolic group includes hepatic encephalopathy [3, 9, 10] and maple syrup urine disease [7] in ruminants. Hepatic encephalopathy had predominant vacuolation in the white matter of the brain stem and at the junction of the gray and white matter of the cerebellum and spinal cord as well as significant hepatic damage [10]. Maple syrup urine disease was suspected of being inherited [7]. The pronounced vacuolation was seen in the cerebrum, brain stem, cerebellum, and spinal cord [7].

The present disease was different from the diseases described above which were inherited or in which there was vacuolation in the spinal cord and cerebrum. But hepatic encephalopathy was somewhat similar to the present disease in that predominant vacuolation was present in the deeper portions of the spinal white matter [9, 10]. Though the exact cause of the present disease remains unknown, some kind of metabolic or toxic disorder might have been associated with it.

The present fatal disease seems to have been caused by clostridial infection, since clostridial organisms were isolated from the parenchymatous organs or intestinal contents of the affected animals with marked hemorrhage throughout the body, and the occurrence ceased following clostridial vaccination of the herd. Infection due to some kinds of clostridial organisms is not known to cause vacuolation in the CNS of calves, though it is reported that the administration of *Cl. perfringens* type D toxin produced vacuolation due to intramyelinic and extracellular edema proceeding to severe malacia in the cerebellar white matter of mice [18]. In view of the present results, it is doubtful that the clostridial infection contributed significantly to the production of the spinal lesions in the present ataxic animals.

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