NOTE *Pathology*

A Neurological Disease with Spongy Degeneration in a Newborn Japanese Black Calf

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ABSTRACT. A Japanese Black calf, 3 day-old male, showed severe ataxia, lateral recumbency, and opisthotonos at the birth. Histopathological examinations revealed severe status spongiosis throughout the central nervous system. Numerous vacuoles within the neuropile varying in size and shape were observed in both formalin-fixed paraffin and cryostat sections. In the lesions, a limited number of spheroids and macrophages were observed within the myelin sheaths with very mild astrogliosis. These vacuoles were negative for both periodic acid Schiff and Sudan black stains. The clinical and histopathological features were almost in conformity with those of bovine maple syrup urine disease (MSUD). Although we could not confirm completely the etiology, congenital hereditary neurological diseases including MSUD are considerable as the possible disease entry in the present case.

KEY WORDS: bovine, maple syrup urine disease, spongy degeneration.

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Spongy degeneration in the central nervous system (CNS) is the most characteristic morphological feature of prion diseases in several animal species. Among them, the diagnosis and disclose of ovine or caprine scrapie and bovine spongiform encephalopathy (BSE) are very important especially in veterinary fields [13]. However, spongiform change is not specific feature for prion diseases, but other several metabolic or hereditary degenerative diseases also cause similar changes in the CNS. In humans, Canavan's disease, autosomal recessive disorder based on N-acetylaspartic acid metabolism alteration, has been known to cause spongy degeneration in the brain [2, 9]. In addition, maple syrup urine disease (MSUD), autosomal recessive disorder due to hereditary metabolic disturbance of branched-chain amino acid (BCAA), results in status spongiosis in the CNS [1]. Even in calves, similar hereditary or metabolic CNS disorders including MSUD and congenital cerebral edema have been reported in the Hereford breed [3–7, 12].

In Japan, the first case of BSE was diagnosed in September 2001. Then, mass-screening system based on a detection of abnormal prion protein (PrP) by ELISA has been adopted at meat inspection and livestock hygiene centers since October 2001. The final diagnoses were based on the results of both Western blot examination and immunohistochemistry. Since the screening system for BSE has applied for all meat cattle and all ill or dead cattle over 24 month-old, hereditary degenerative disorders other than prion diseases might not be serious differential diagnosis to rule out. Besides, when very young calves developed spongy degeneration in the brain, these diseases could be very important. In addition, status spongiosis in the CNS may be induced by heavy metal poisoning such as triethyl tin [10]. It has been also known that spongiosis-like vacuolar changes are often produced by inadequate tissue processing for paraffin sections.

The present study describes pathological features of a Japanese Black calf with severe congenital neurological signs, which were characterized by status spongiosis in the CNS and discusses possible etiology.

A newborn Japanese Black calf, 3 day-old male, complaining astasia was presented to the Department of Veterinary Anatomy, Miyazaki University. The case showed clinical signs including ataxia, lateral recumbency, opisthotonos, and tonic muscular contractions. Necropsy was performed immediately for pathological examinations. Except for ocular opacity and meningeal congestion, no significant gross lesions were observed. For histopathological examinations, tissue samples of the brain and eyes were collected. The brain was fixed with 10% neutral buffered formalin and the eyes were fixed with methanol-Carnoy's solution, respectively.

For routine histopathology, paraffin-embedded sections of 6 μm thick were made and were stained with hematoxylin and eosin (HE). Cryostat sections were prepared from formalin fixed brain tissues for periodic acid Schiff (PAS) and Sudan black stains. Selected paraffin sections were also stained with Luxol fast blue (LFB) for myelin. To evaluate the degree of vacuolar changes in each brain region, the mean square of vacuoles was measured by an image analysis system (WinRoof, Mitani Corporation, Tokyo, Japan). The sections from the cerebral cortex, hippocampus, mesencephalon, cerebellar white matter, medulla oblongata, and spinal gray matter, were used for image analysis. For statistical analysis, the mean number from 5 randomly selected filed in each region was examined by *t*-test ($P<0.01$). Immunohistochemistry was performed using Envision Polymer reagent (DAKO Japan, Kyoto, Japan). The primary antibodies were rabbit antisera against glial fibrillary acidic protein (GFAP, prediluted, DAKO Japan), and mouse monoclonal antibody against neurofilament (NF, prediluted, DAKO Japan). Lectin histochemistry was performed using biotin-labeled lectin *RCA-1* (EY laboratories Inc., San

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Table 1. The distribution and degree of vacuolar change in the CNS

CNS regions	vacuolar change
Cerebral cortex and white matter	+++
Hippocampus	++
Striate body	++
Diencephalon	++
Mesencephalon	
gray layer of superior colliculus	–
central gray matter	+
oculomotor nucleus	++++
Medulla oblongata	
dorsal vagal nucleus	++
solitary nucleus	+++
spinal trigeminal nucleus	+
hypoglossal nucleus	+
cuneate nucleus	+++
gracile nucleus	–
olivary nucleus	+
pyramidal system	+++
Cerebellum	
cortex	–
white matter	++++
Spinal cord	
dorsal horn	+
lateral horn	++++
cornicle	+
white matter	–

The degree of the lesions represents according to the percentage of vacuolar space. –; no vacuolar change, +; limited number of vacuoles (<5%), ++; mild spongy change (5–10%), +++; moderate spongiosis (10–15%), and ++++; severe spongiosis (15%<).

Mateo, CA, U.S.A.) and avidin-biotin peroxidase complex method (ABC, PK-4000, Vector Laboratories, Burlingame, CA, U.S.A.). The reaction products were visualized by 3,3'-diaminobenzidine (Sigma, St. Louis, MO, U.S.A.).

Histopathological examinations revealed severe spongy degeneration distributed throughout the CNS. The profile of the vacuolar lesion in each area is summarized in Table 1. Numerous vacuoles within the neuropile varying in size and shape were widely distributed in the cerebral cortex and white matter, hippocampus (Fig. 1a), striate body, diencephalon, mesencephalon (Fig. 1b), cerebellar white matter, medulla oblongata (Fig. 1c), spinal gray matter, and retina (Fig. 1d). These vacuolar changes were prominent especially in the oculomotor nucleus of the mesencephalon, cerebellar white matter, and lateral horn area of the spinal cord. In these regions, neurons were compressed by vacuoles with mild degenerative changes of the neurons and axonal spheroid. In the eyes, vacuolar changes were located in the ciliary retina, inner granular, outer granular, and nerve cell layers. Among those areas, the lesion was most prominent in the ciliary retina.

The vacuolar changes were observed frequently in the deep cerebral cortex and white matter, and molecular layer of the dentate gyrus, while the lesions were less prominent as compared with these in the mesencephalon, cerebellum, and spinal cord. The degree of vacuolar changes in each region is provided in Fig. 2. Statistically, there were significant difference ($P<0.01$) among each other except for between the hippocampus and medulla oblongata, the mesencephalon and cerebellar white matter, the mesencephalon and spinal gray matter, and the cerebellar white matter and spinal gray matter. In the mesencephalon and spinal gray matter, the maximum size of the vacuole was up to 130 μm in diameter, whereas in the other regions, it was up to 60 μm. There were no distinct intracytoplasmic vacuoles in the neurons. In the lesions with spongy degeneration, small number of macrophages infiltrated within the neuropile with mild astrogliosis. In addition, vacuoles were usually lined by myelin sheaths in the cerebral and cerebellar white matter by LFB stain. These vacuoles also could be observed in formalin fixed cryostat sections and were negative for both PAS and Sudan black stains. In any brain regions, atypical astrocytes were absent, and there were a few ischemic neurons.

The characteristic findings of the present case were spongy degeneration. Spongiosis-like vacuolar changes in the brain tissue are often observed as an artifact by inadequate tissue processing for paraffin sections. However, vacuolar changes in this case were not considered as an artifact because of the significant clinical signs and the presence of reactive macrophages and axonal spheroids in the lesions. In addition, the present vacuolar changes were found both paraffin and cryostat sections of the brain and paraffin sections of the eyes, which processed by different fixative, methanol-Carnoy's solution. These facts indicate the vacuolar changes in the present case might be significant pathologic lesions to explain the clinical signs.

Spongy degeneration in the CNS is the most characteristic histological feature of prion diseases including ovine or caprine scrapie and BSE caused by accumulation of PrP [8, 13]. These prion diseases take long incubation period approximately five years until the onset. Histopathologically, there are intracytoplasmic vacuoles in the neurons and axons, and the neuropile of gray matter presents status spongiosis with mild to moderate astrogliosis. Some authors have reported the consistency of the vacuolar lesion profiles among British cattle with BSE [11, 14], and those of the first BSE case in Japan [8]. Vacuolar lesions were mainly in the brain stem including mesencephalon and medulla oblongata, and there were no lesions in the cerebrum. On the contrary, in the present case, the calf affected at the birth. Moreover, the distribution patterns of the vacuolar lesions were inconsistent with those of BSE cases. Therefore, the possibility of BSE can be excluded.

In Hereford calves, MSUD and congenital cerebral edema also cause wide spread vacuolation or status spongiosis in the CNS have been reported in Australia, New Zealand and, United Kingdom [12]. These congenital disorders present common clinical course that calves are affected in the first week of life with severe neurological signs of recumbency and opisthotonos. Congenital cerebral edema was first reported by Jolly in New Zealand [7]. Histopathological features are characterized by hypomyelinogenesis,

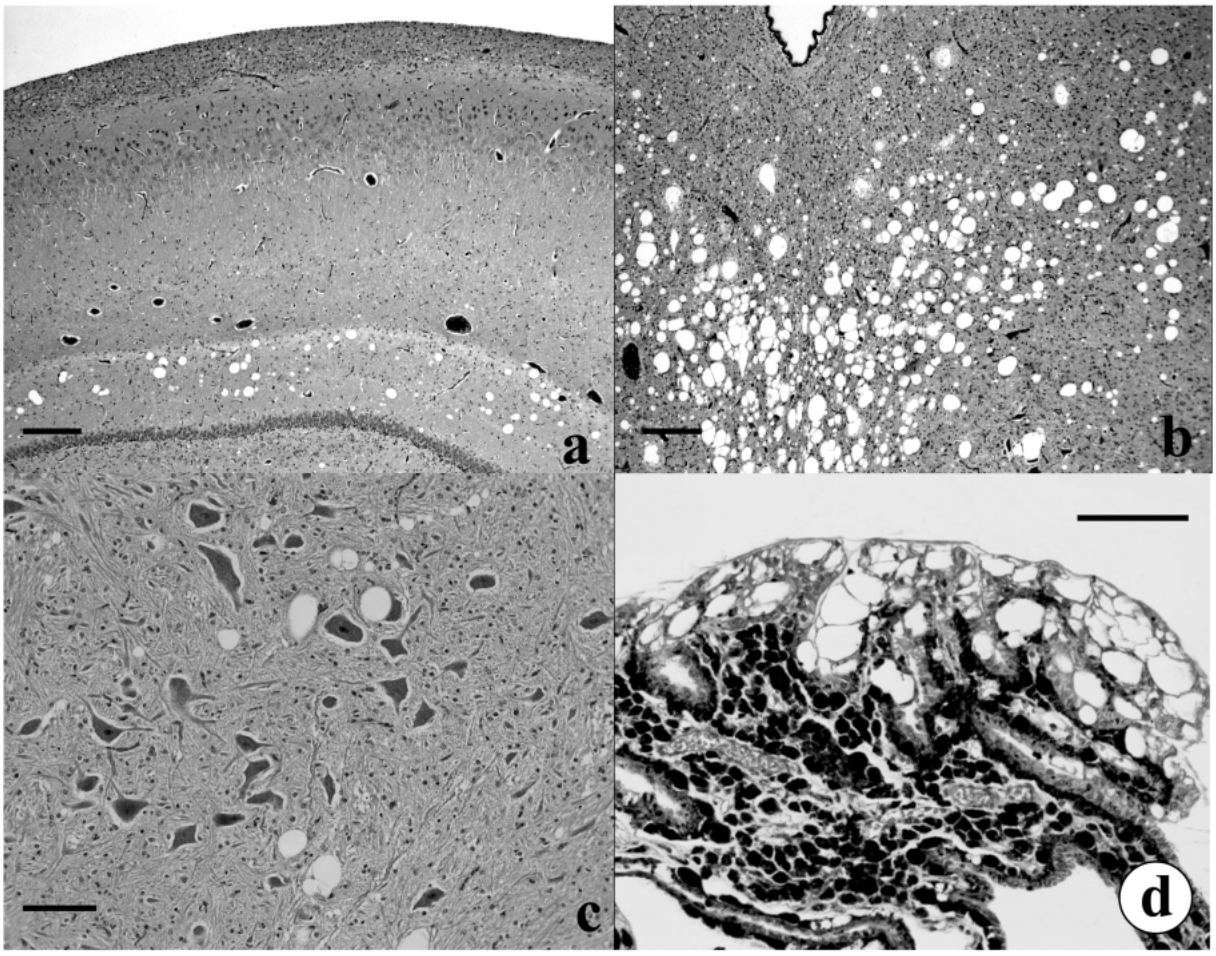

Fig. 1. (a) Mild vacuolar changes in the molecular layer of the dentate gyrus. HE stain. bar = 200 μm. (b) Severe spongiosis in the oculomotor nucleus of the mesencephalon. The maximum size of the vacuoles was up to approximately 130 μm in diameter. HE stain. bar=200 μm. (c) Vacuoles within the neuropile in the medulla oblongata dorsal vagal nucleus. HE stain. bar=100 μm. (d) Vacuolar change in the ciliary retina. HE stain.bar=100 μm.

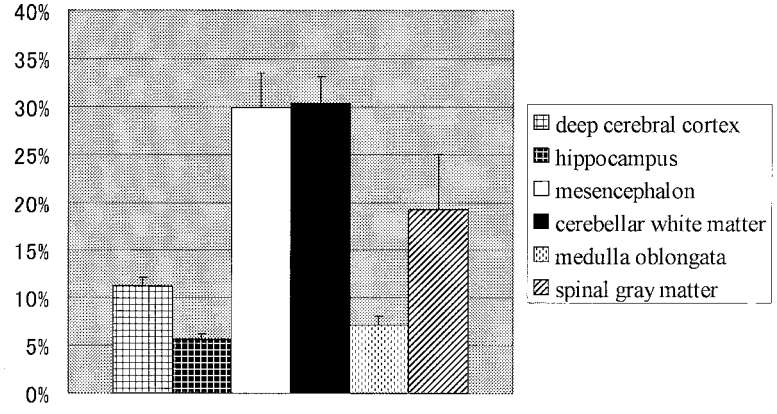

Fig. 2. The mean square of vacuoles in each brain region, deep cerebral cortex (11.8%), hippocampus (5.6%), mesencephalon oculomotor nucleus (29.9%), cerebellar white matter (30.4%), medulla oblongata dorsal vagal nucleus (7.1%) and spinal cord lateral horn area (19.2%).

and hydropic degeneration of astrocytes and oligodendrocytes resulting in status spongiosis in the CNS. However, there were no significant histological changes in both astrocytes and oligodendrocytes in this case. These histological findings indicated that the present case could be distinguished from congenital cerebral edema. MSUD is metabolic disorder of BCAA based on branched-chain alpha-keto acid dehydrogenase complex (BAKAD complex) deficiency, and the investigation of bovine MSUD has advanced to genotyping in Australian Poll Herefords [6]. The accumulation of BCAA leucine, isoleucine, valine, and their respective keto acids are determined in serum, plasma, cerebrospinal fluid, and formalin fixed CNS tissue. Consequently, spongy degeneration is appeared within the CNS as the secondary change due to spongiform myelinopathy and vacuolar change is most prominent in cerebellar white matter, spinal gray and adjacent white matter. The clinical course and histopathological lesions in this case were almost in conformity with those of MSUD. As far as we know, there is little information concerning vacuolar change in the retina with MSUD. The retinal vacuolar lesions in the present case supposed to appear in the same manner of those in the CNS because of the embryologic origin of these tissues. Although we could not confirm the etiology completely, congenital hereditary neurological diseases including MSUD are considerable as the possible cause of spongy degeneration in the present case. Further studies to identify the etiology of this case will be needed.

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