Segmental spinal dysgenesis with caudal agenesis in a Holstein calf

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ABSTRACT. A rare complex dysraphic malformation, comprising segmental spinal dysgenesis with caudal agenesis, was found in a Holstein calf that was unable to stand and was slightly short at the lumbosacral spine with taillessness. The thoracolumbar and sacrococcygeal regions of the midline axial segments showed severe deformities. In the spinal cord, the thoracolumbar region showed severe constriction with myelodysplastic changes, and the sacrococcygeal region showed dorsoventral separation with connection to a neural mass. In the spine, vertebral anomalies according to the degree of the segmentation error were confirmed. The cervical and thoracic segments also showed milder dysraphic changes. These changes suggest a multisegmental causal insult impairing the early embryonic notochord. This represents the first bovine case definitively confirmed morphologically.

KEY WORDS: caudal agenesis, complex dysraphic malformation, Holstein calf, segmental spinal dysgenesis

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Spinal malformations and spinal cord malformations are collectively termed spinal dysraphisms. Several varieties of spinal dysraphism exist, classified by neuroradiological features and embryological correlations [31]. These can be broadly categorized into open spinal dysraphisms (OSD) characterized by abnormal exposure of nervous tissues through a skin defect, and closed spinal dysraphisms (CSD) characterized by continuous skin coverage over the underlying malformation [24–26]. In cattle, the former are often reported as spina bifida aperta and the latter as spina bifida occulta [5, 8]. In addition, some rare cases of CSD have been reported, such as segmental hypoplasia in Holstein [3, 28] and Japanese Black [14], diplomyelia in Holstein [4], Japanese Black [18, 27, 33] and crossbreed (Holstein × Belgian Blue) [29] and diastematomyelia in Japanese Brown [30]. We describe herein a rare case of complex dysraphic malformation as a CSD in a calf, in the form of segmental spinal dysgenesis with caudal agenesis. Morphological changes of the malformed spinal cord and vertebrae were examined, and the pathogenesis was discussed from an embryological perspective.

The subject was a male Holstein calf delivered by a cow that received a fertilized ovum on November 11, 2011. After birth, he was unable to stand due to paresis and slightly short spine in the lumbosacral region with taillessness and rapid respiration. The calf was euthanized at 4 days old on the request of the owner, and postmortem examination was performed. The calf weighed 32 kg at necropsy, indicating growth retardation. This was the third delivery of the 4-year-old dam, which had received annual vaccinations since birth against arboviral infections to prevent congenital abnormalities and had not been administered any drugs during the pregnancy. No common ancestor was evident between the maternal and paternal lines of the calf, and no similar cases were evident in the lineage.

During necropsy, the spinal column was observed to show a kyphoscoliotic curvature (KSC) from thoracic vertebra (T) 12 to lumbar vertebra (L) 4 and was missing caudal to sacral vertebra (S) 5. The spinal cord showed a constriction between L1 and L5, with particularly severe constriction at L2-L4 (diameter: approximately 3 mm) (Fig. 1). In the constricted region, the nerve roots in each segment were either missing or markedly reduced in number. At the L6-S2 level, the cord was bulky and thickened, and the conus medullaris was located more caudally (about the S3 level). The cauda equina was not fully confirmed. Moreover, incomplete dorsoventral duplication of the spinal cord within a single dural covering at the termination (about S4 level), corresponding to the tip of the conus medullaris and filum terminale, was identified. A neural mass was connected to the ventral part of the duplicate cords with a thin pia mater (Fig. 1).

Transverse sections of the cervical and thoracic spinal cords rostral to the KSC revealed the cavitations within the

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neuroparenchyma in some segments (Fig. 2A). Histologically, these cavities of varying size affected both white and gray matters, and the walls of the cavitations were lined by poorly stained parenchymal glial and/or nerve fibers, indicating syringomyelia. In these areas, dilation of the central canal, indicating hydromyelia, was also seen. Occasionally, the syringomyelic cavity (SC) and hydromyelic cavity (HC) were connected. At constricted regions of spinal cord (T12-L5) mostly corresponding to the KSC level (Fig. 2B), histological changes, such as HCs and SCs, reduction in size or absence of the central canal, poor delineation of gray matter and absence of the dorsal and ventral gray horns, absence and partial penetration of the dorsal septum, and a ventral median fissure were confirmed. However, HCs and SCs were rare in the severely constricted segments (L2-L4). Caudal to the KSC, whereas the caliber of the bulky cord was hypertrophically large and associated with HCs and SCs, the H-shaped appearance of gray matter and median formation of the dorsal septum and ventral fissure were mostly maintained (L6, S1). In the terminal portion (S2-S4 level), a thin, horizontal, fibrous septum bisected the spinal cord into dorsoventrally asymmetrical duplicate cords. Each cord, with occasional HCs and SCs, comprised a slender, elliptical, ependyma-lined canal, the neuroparenchyma with ambiguous division of the gray and white matters and stray pia mater-like septa are discernible. HE stain. Bar=1 mm

Fig. 1. Constriction of the lumbar spinal cord from L1 to L5 with caudal bulky cord. Bar=5 cm. In severely affected segments (L2-L4), the diameter of the cord is 3 mm. Inset: Caudal terminal portion (about S4 level) after fixation. Bar=5 mm. The ventral part of the duplicate cords and a neural mass (NM) are connected with the thin pia mater (dotted arrow). *: Some artificial damage was incurred on removal from the spinal canal.

Fig. 2. Transverse and histological appearances of affected segments of the spinal cord. A: Some small cavities (small arrowheads) are formed in the neuroparenchyma at C3. Bar=1 cm B: Poor delineation of the gray matter (GM), reduction in size of the central canal (arrowhead), absence of the dorsal septum and penetration of fibrous connective tissue like a ventral fissure (arrows) are shown at the constricted segment (L2). Azan stain. Bar=1 mm C: A dorsoventrally asymmetrical set of duplicate cords is indicated at the terminal portion (S2 level). In each of the duplicate cords bisected by a thin fibrous septum, a slender, elliptical, ependyma-lined canal (white arrowhead), ambiguous division of the gray and white matters and stray pia mater-like septa are discernible. HE stain. Bar=1 mm
large, ependyma-lined canal with flat shape lengthwise, discriminable gray and white matters, and dorsal septal and/or ventral fissural penetrations of the pia mater.

Serial sections of the caudal terminal segments (Fig. 3) confirmed a partial connection between the duplicate cords. The neural mass was connected to the ventral duplicate cord by thin, fibrous neuronal tissue. The ependyma-lined canal in the dorsal cord was continuous with the central canal of the anterior segments, and another isolated, small, elliptical/spherical cavity was also formed. In the ventral cord, the ependyma-lined canal was cylindrical with an anterior tapered end and some irregular posterior branches. Furthermore, a dorsal branch connected to the ependyma-lined canal continuous with the central canal of the anterior segments through the partial connection between duplicate cords.

From a macerated specimen for identification of vertebral anomalies, fusion of the right vertebral arches (T2, T3) and fusion and deformation of vertebral bodies (T5, T6) with absence of the left vertebral arch (T5) were seen rostral to the KSC. Hemivertebrae at T12 fused to L1 due to loss of T13, mild wedged vertebrae between L1 and L4 and fusion of the vertebral body between S2 and S4 are found. Inset: Anterior aspect of L1 and L2. Bar=2 cm. Vertebral foramen of L2 is markedly narrower than that of L1.
coagulation were also seen. Neutralizing antibodies against teratogenic arboviruses (Akabane, Chuzan and Aino) in serum samples were titrated to 256 for Akabane, 64 for Chuzan and 32 for Aino.

Generally, abnormal development of the spinal cord is referred to as myelodysplasia [19]. Hypoplasia is one of the major categories of myelodysplasia and is defined as reduced development of segments of the spinal cord [13]. Histological changes at the severely constricted lumbar segments of the spinal cord in the present case, such as dorsal and ventral septal disturbances, aberration of the central canal and structural anomalies of the neuroparenchyma involving distortion of the gray matter, are consistent with the characteristics of myelodysplasia [19] and indicate poor and abnormal development. From the range and degree of anomalous changes, the myelodysplasia in the affected region would be diagnosed as segmental hypoplasia. Compared with similar reported cases [3, 14, 28], the grade and range of the constricted part in the present case were more severe. In the spinal column of these cases, although mild curvature [14] and mild lordosis [3] were present, markedly deformed vertebrae were not observed. In the present case, segmental vertebral malformations including hemivertebrae, fusion and partial agenesis of the vertebral body and/or arches are associated in the affected regions. Defects of segmentation in the spine, such as perosomus elumbis [10, 12], complex vertebral malformation [2, 10, 21], short spine lethal [10], brachyspine [1] and spinal curvature [22], have been reported in cattle, but no cases of myelodysplasia have been confirmed except in perosomus elumbis. In humans, segmental spinal dysgenesis (SSD) is characterized by focal agenesis or dysgenesis of the lumbar or thoracolumbar spine, with focal abnormality of the underlying spinal cord and nerve roots [26, 32]. SSD is categorized by complex dysraphic states in a closed spinal dysraphism without mass and is morphologically found as aplasia or hypoplasia of the spinal cord with vertebral malformations, such as agenesis or dysgenesis of the vertebral body, deformation of the vertebral arches and spinal canal stenosis at the affected segments [24, 31]. The morphological changes of the spine and spinal cord in the thoracolumbar segments (KSC level) in the present case would be compatible with SSD.

In the caudal duplicated terminal segments of the spinal cord, a thin fibrous connective tissue bisected the spinal cord dorsoventrally into two separate compartments. Regional separation of the spinal cord by a thin fibrous septum within a single dural tube would resemble diplomyelia, and the histological appearance of the duplicate cords was similar to the hemicord in split cord malformation [23]. On the other hand, since the split cord malformation, such as diplomyelia and diastematomyelia, would be considered to originate from splitting of the notochord and the overlying neural plate due to failure of the midline integration of the two paramedian notochordal anlagen along the rostral lip of the primitive node [23, 31], duplication of the spinal cord would be formed laterally and the affected site would be rostral to the coccygeal segments that ultimately comes to lie opposite the primitive pit. The caudal duplicated terminal segments of the spinal cord in the present case would not be considered to be embryologically correlated to the split cord malformation and would result from an insult to the secondary neural tube derived from the tail bud. The tail bud is a mass of pluripotent tissue from which the terminal portion of the spinal cord develops and is also responsible for development of the caudal notochord [9]. In the caudal spine, caudal vertebral malformations, such as absence of the posterior segments from S5 and fusion of the vertebral bodies between S1 and S5, were also seen. Caudal anomalies of the spine and spinal cord in the present case would thus be related to the developmental error of the tail bud and are compatible with caudal agenesis (CA), which refers to complex anomalies including partial and complete absence of the caudal vertebrae together with corresponding regions of the caudal neural tube [7]. In cattle, although sacrocaudal agenesis [16] and tailless [11] have been reported, the histological appearance of the spinal cord has not been described.

From the above mentioned description of the main pathological changes, the malformation in the present case of the spine and spinal cord at two separate segmental levels, i.e., thoracolumbar and sacrococcygeal regions, was diagnosed as a rare associated anomaly of SSD and CA, which represents the first bovine case definitively confirmed morphologically, to the best of our knowledge. SSD and CA are considered as abnormalities depending on notochord formation during gastrulation, and probably represent two faces of a single spectrum of segmental malformations involving the spine and spinal cord [24, 32]. They differ from an embryological perspective in terms of the segmental location of the derangement along the longitudinal axis of the embryo. In SSD, the intermediate segment is involved, as opposed to the caudal segment in CA [24, 32]. During gastrulation, prospective notochordal cells wrongly specified in terms of their rostrocaudal position are eliminated, so fewer or even no cells at all will form the notochord or tail bud depending on the segmental level of the abnormality [31]. From an embryogenetic point of view in the present case, depopulation of the prospective notochord in the thoracolumbar segments (KSC level) would induce neurulation of a depauperate neural plate, in turn resulting in severe hypoplasia of the spinal cord, and would affect development of the spinal column to result in vertebral malformations. Simultaneously, the causal insult would impair the terminal segments derived from the tail bud and would result in the dorsoventral duplicate cords and neural mass with aberrant canalization. Furthermore, the mild dysraphic changes at some segments of the cervical and thoracic regions might suggest multisegmental derangement of the early embryonic notochord. Other defects not involving the axial segments, such as fused kidney and extra-lobulated liver, might reflect impaired induction from the depopulated notochord to the affected organs.

Some cases of myelodysplasia have been associated with fetal Akabane infection [19]. However, no lesions in this case appeared associated with Akabane infection, such as non-suppurative encephalitis [15] or arteriothrombosis-hydranencephaly syndrome, which is a recognized sequela to infection during fetal development [17]. Detection of antibodies against teratogenic arboviruses (Akabane, Aino and Chuzan viruses) in calf serum were very likely attributable to colostrum from the vaccinated dam. Intrauterine infections with bovine viral diarrhea virus induce a number of fetal abnormalities, including central nervous system malformations and skeletal deformities [19, 20]. However, no objective evidence was obtained of this viral infection, such as characteristic lesions including cerebellar hypoplasia, cerebral defects, ocular anomalies, bent front leg or jaw/head abnormalities, or clinical signs involving the respiratory and digestive systems in the dam during pregnancy. A simple genetic cause was eliminated from the
family line in the absence of a common ancestor or similar cases in the lineage. The etiology of SSD and CA in the present case remains to be fully defined. Modern findings for human neural tube defects, which support multi-gene predispositions together with a role for environmental factors, such as the diabetic milieu or folate status [6], have attracted our attention and will represent a future subject of investigation.

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