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NOTE

Anatomy

Tethered spinal cord related to caudal spinal dysraphism in a tailless Holstein calf

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ABSTRACT. A rare dysraphic caudal spinal anomaly, or caudal agenesis, comprising a tethered spinal cord, was found in a tailless Holstein calf that presented ataxia and paresis with analgesia of the hind limbs. The gently and slimly tapered conus medullaris was poorly formed between S2 and S3 which indicated that it was lying more caudally. The caudal end of the filum terminale adhered to the inner periosteum of the vertebral arch at S4, which is compatible with tethering of the spinal cord. The dysraphic changes from the secondary neurulation error and the longitudinal deranged cord morphology that may have been caused by the caudad traction due to tethering were confirmed. This represents the first bovine case with definitive morphological confirmation.

KEY WORDS: caudal agenesis, closed spinal dysraphism, Holstein calf, tailless, tethered spinal cord

A tethered spinal cord (TSC) is best defined as an abnormal attachment of the spinal cord to the tissues that surround it [1]. In humans, the spinal cord is most frequently tethered in the lumbosacral region [1]. The filum terminale in these cases is often abnormal with adhesions to the adjacent, dura, and a lower lying conus medullaris than normal is characteristic. This state is primarily related to some closed spinal dysraphism, especially at caudal regions, such as spinal lipomas, tight filum terminale, split cord malformations, and the caudal regression syndrome, and it can be secondary to scarring or formation of a dermoid in the aftermath of myelomeningocele repair [37]. The distinctive clinical symptoms and signs reflecting both motor and sensory neuron dysfunction, which are induced by the excessive tension on the spinal cord due to tethering, are often referred to as tethered cord syndrome. In cattle, some cases of closed spinal dysraphism such as segmental spinal dysgenesis with caudal agenesis [36], segmental hypoplasia [6, 18, 33], diplomyelia [7, 20, 30, 31, 34] and diastematomyelia [35] have been reported. However, there have been no such reports of TSC.

Recently, TSC was observed in a tailless Holstein calf in the Kumamoto Prefecture of Japan. In this paper, the morphological changes of the caudal spinal cord are presented, and the pathogenesis is discussed from an embryological perspective.

The subject was a male Holstein calf delivered on May 22, 2015. After birth, he was unable to stand, there was no tail, and he showed paresis with analgesia of the hind limbs. The calf was euthanized at 3 days of age by a physical method (electrocution) [4] under xylazine sedation at the request of the owner, and a postmortem examination was performed. This study was approved by the Institutional Animal Care and Use Committee at Tokai University (approval number 153042). The calf weighed 38.5 kg at necropsy. This was the second delivery of the 2-year-old dam, which had not received annual vaccinations since birth against arboviral infections to prevent congenital abnormalities and had not been administered any drugs during the pregnancy. The dam had birthed a normal calf in the first delivery. The sire was not a carrier of any of the following recessive disorders: bovine leukocyte adhesion deficiency, complex vertebral malformation, or brachyspina. No common ancestor was evident between the maternal and paternal lines of the calf.

During necropsy, the spinal column was missing caudal to sacral vertebra (S) 5 and fusion was observed from S3 to S5. On carefully removing the vertebral arch of each segments from the whole spinal column, adhesion of the caudal end of the filum terminale to the inner periosteum of the vertebral arch at S4 was confirmed (Fig. 1A). On careful observation after fixation, the lumbar intumescence appeared to be narrow, and the caudal extremity of the spinal cord between S2 and S3 level tapered gently and slimly, indicating poorly elongated formation of the conus medullaris, to connect to the fibrous filum terminale. The cauda equina was not fully confirmed. The transverse appearance of the parenchyma from the cervical to the lumbar segments was often more flattened and somewhat square-like (Fig. 1B), and it was also appeared to be vertically lower than controls based on the calculation of the ratio of the dorsoventral to the left-right diameters at selected segments of the spinal cord (Table 1). An annular constriction was formed at the caudal tapering cord at the about S2 level. The shortened filum terminale of about 2 cm extended to adhere to the unossified cartilage piece of the fourth sacral vertebral arch (Fig. 1C).

Histologically, at the vertically lower segments from the cervical to lumbar regions, although the neuroparenchyma including the

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central canal seemed to have been compressed oblongly, the compartmentalization of the gray and white matters with median septa was clear and no pathological changes were seen. However, around and at the posterior regions of the annularly constricted level, various lesions were confirmed and the characteristic histological changes are shown in Fig. 2. The neuroparenchyma formed irregular angular shapes such as polygons (Fig. 2A), trapezoids (Fig. 2B), and triangles (Fig. 2C) with the poorly formed dorsal septum and the ventral fissure. The gray matter and white matter were irregularly demarcated and formed crack-like cavities that were lined by poorly stained glial and/or nerve fibers, indicating syringomyelia (Fig. 2A–D). The central canal was also in bizarre states, such as connecting to a syringomyelic cavity (Fig. 2A), lobular or outpouching shapes (Fig. 2B) and an increase in the number of central canals (Fig. 2C). Furthermore, the terminal end of the caudally tapering spinal cord and the fibrous filum terminale were duplicated (Fig. 2D).

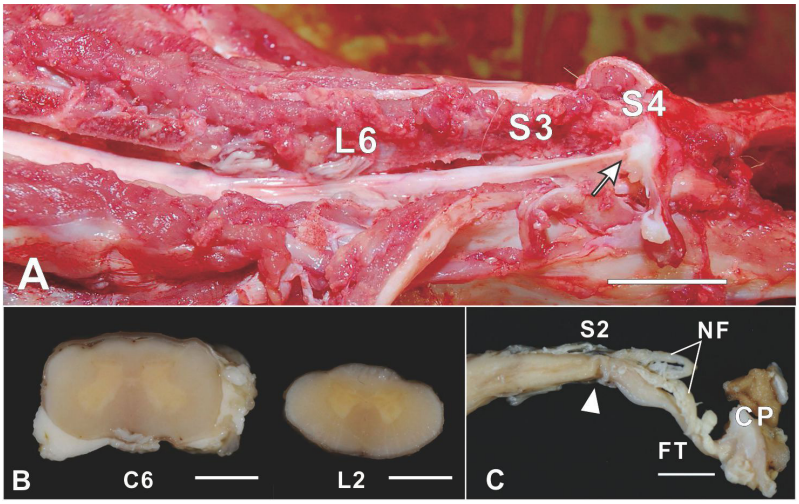

Fig. 1. Gross appearance of the tethered spinal cord. A) The caudal end of the filum terminale adheres to the vertebral arch of S4 (arrow). The conus medullaris is poorly formed at the S3 level and elongated. Bar=2 cm. B) Transverse sections are often more flattened and somewhat square-like from the cervical to the lumbar segments. Bar=5 mm. C) Annular constriction (arrowhead) is seen at the terminal caudal region. Bar=1 cm. CP: Unossified cartilage piece removed from the fourth sacral vertebral arch. FT: filum terminale. NF: nerve fiber.

The serial sections from the caudal extremity of the spinal cord to the filum terminale confirmed the relationship between the appearance of the spinal cord and the distribution of the central canal (Fig. 3). The central canal closed at the annular constricted part as a cul-de-sac, and, after that, the fragmented canals bifurcated to each terminal duplicated cord. Each terminal end of the duplicated cord connected the duplicated fibrous filum terminale to the periosteal membrane around the unossified cartilage tissue of the fourth sacral vertebral arch.

From the maceration specimens of the sacral vertebrae, block vertebra between S2 and S4 were confirmed. S5 and its caudal coccygeal vertebrae were missing. No other organs were seen in the gross and microscopic lesions.

In cattle, the end of the spinal cord is at the level of S2 at two months of age [9], and it reaches into the caudal half of the sacrum in young calves [12]. The conus medullaris in the control (10-day-old Holstein calf) was located from L6 to S1. The poorly formed conus medullaris at the S2 and S3 levels in the present case appeared to be lying more caudally, and normal ascent of the spinal cord would have been prevented due to the adhesion of the caudal end of the filum terminale to the inner periosteum of the fourth sacral vertebral arch. Therefore, these changes would be compatible with TSC.

The tethered cord causes lumbosacral neuronal dysfunction that could be due to impairment of mitochondrial oxidative metabolism under constant or intermittent cord stretching [38]. The clinical signs such as astasia and paresis with analgesia of the hind limbs in the present case might have been due to progressive motor and sensory changes caused by abnormal cord stretching. On the other hand, the distant neuroanatomic defects in cases of spina bifida depend on fixation of the spinal cord at an abnormally low level with abnormal stretching of the cord [19, 22]. With experimental traction at the conus medullaris-cauda equina region, the maximum cord elongation occurs in the lumbar region, some occurs in the thoracic area, and minimal to no elongation occurs in the cervical region [29]. Although the transverse appearance and histological changes of the affected spinal cord were not fully described in previous reports, the longitudinally deranged cord morphology in the present case, such as the vertically lower segments from the cervical to the lumbar region and the elongation of the gently tapered conus medullaris, may have been caused by the caudad traction due to tethering. It is also thought that the increased tension and aberrant stretching of the spinal cord during the fetal period would affect the architecture of the neuroparenchyma. Consequently, the appearance of the transverse section of the terminal spinal cord would seem to have changed to a lower height with irregular angular shapes. These changes were not seen in the reported cases of bovine spinal dysraphism [6, 7, 18, 20, 30, 31, 33–36].

Furthermore, the caudal vertebral anomalies, such as the mild deformed sacrum and missing coccyx are compatible with the most consistent finding in caudal agenesis (CA), which refers to the malformations of structures derived from the caudal region of the embryo [3, 10, 14]. CA involves abnormal or incomplete formation of caudal elements of the embryo and arises from problems with canalization of the caudal neural cord (the secondary neural tube) or in the process of retrogressive differentiation during the ascent of the conus medullaris. Because the filum terminale forms as a glioependymal strand during retrogressive differentiation, CA often leads to an elongated and tethered conus [1]. A congenital error in the canalization of the caudal bud of the spinal cord would cause adhesion of the filum to adjacent structures, preventing the relative ascent of the conus medullaris [28]. Therefore, it would be expected that the adherence of the filum terminale to the periosteum of the fourth sacral vertebral arch is induced by the faulty tissue separation of the caudal elements derived from the tail bud during secondary neurulation. Histological changes at the caudal extremity of the spinal cord, which were observed as poor formation of the dorsal septum and of the ventral fissure, the irregular demarcation of the gray matter and white matter with a syringomyelic cavity in the neuroparenchyma, and the bizarre states of the central canal, such as the

Table 1. Diameters of the transverse sections of seleted spinal cord segments

Selected segments	TSC[a)]			Controls[b)]											
				1			2			3			4		
	DV[c)]	LR[c)]	DV/LR	DV	LR	DV/LR	DV	LR	DV/LR	DV	LR	DV/LR	DV	LR	DV/LR
C3	8.6	12.8	0.67	10.5	12.5	0.84	10.6	13.5	0.79	10.4	11.9	0.87	10.0	13.0	0.77
C6	8.0	13.6	0.59	10.7	17.7	0.60	11.3	16.7	0.68	12.1	16.4	0.74	10.0	15.9	0.63
T6	6.5	10.8	0.60	9.1	11.1	0.82	8.9	11.2	0.79	10.0	10.8	0.93	9.2	11.9	0.77
L2	7.0	12.1	0.58	9.7	12.8	0.76	9.3	12.7	0.73	10.1	12.1	0.83	8.8	12.8	0.68
L6	8.3	14.5	0.58	10.3	16.6	0.62	9.9	16.4	0.60	13.1	15.9	0.82	9.6	15.6	0.62
S3	7.8	11.6	0.67	6.3	7.8	0.81	5.2	6.1	0.85	6.5	7.8	0.83	6.4	7.2	0.89
Full length of the spinal cord	75 cm			78 cm			75 cm			68 cm			81 cm		

a) TSC: tethered spinal cord (the subject). b) Because it is difficult to prepare the normal spinal cord of a healthy calf of the same age in the same breed as the case of TSC, the following controls without gross lesions of the spinal cord from the autopsied calves at Tokai University are provided. The length of the spinal cord is mostly the same as TSC in Nos. 1 and 2, and is slightly different from TSC in Nos. 3 and 4. 1: Japanese Black, male, 55 kg, 50 days old, diagnosed with pneumonia and astasia. 2: Japanese Brown, male, 55 kg, 21 days old, diagnosed with microphthalmia and a tibial fracture. 3: Holstein, male, 39 kg, 2 days old, diagnosed with a cardiac anomaly and a urachal rest. 4: Holstein, female, 66 kg, 90 days old, diagnosed with a cardiac anomaly and an abdominal hernia. c) DV: dorsoventral diameter [mm], LR: left-right diameter [mm].

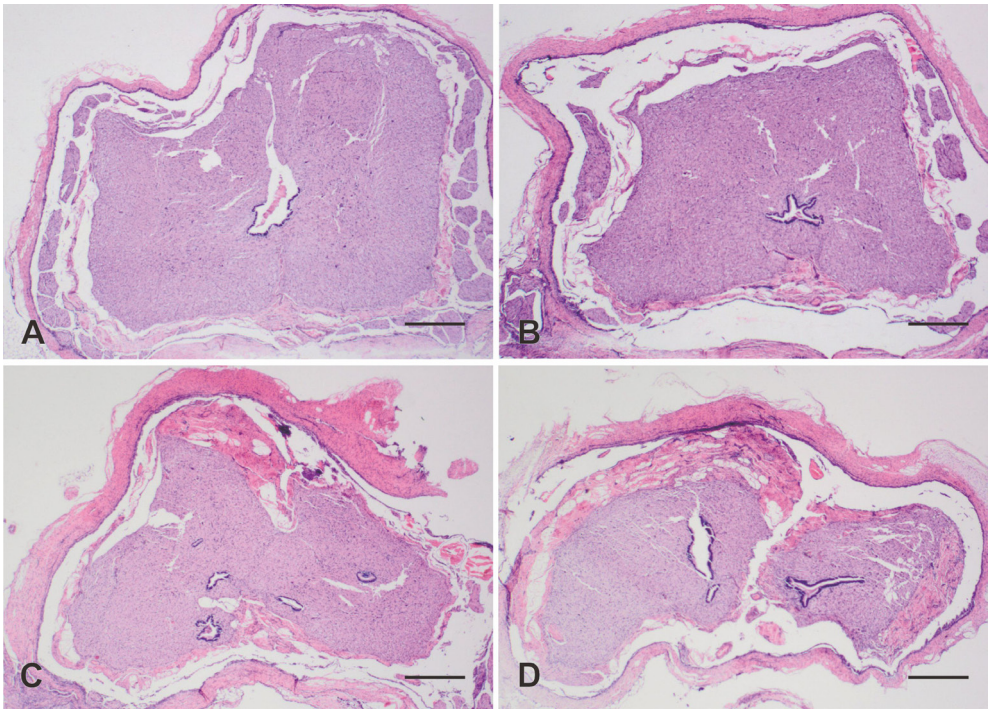

Fig. 2. Histological appearance of transverse sections of the caudal tapering spinal cord around and at posterior regions of the annular constricted portion. Each photomicrograph, which is the same as the site of the corresponding parts in Fig. 3, shows various degree of the syringomyelic crack-like cavities within the neuroparenchyma. Abnormal shapes of the intradural spinal cord and various irregular states of the central canal are also seen: A, polygonal appearance with irregular central canal connected to syringomyelic cavities; B, trapezoidal appearance with lobulated central canal; C, triangular appearance with multiple central canals; D, duplicated cords within the dura mater. Bar=500 µm.

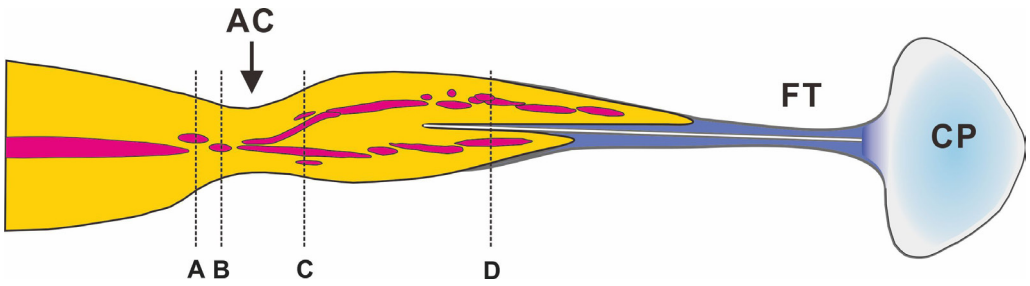

Fig. 3. Dorsal view illustration of the relationship between the duplicated terminal spinal cord and the filum terminale (FT) with the distribution of the abnormal central canal (magenta). The duplicated fibrous filum terminale adheres to the periosteal membrane around the unossified cartilage piece (CP) removed from the fourth sacral vertebral arch. The fragmented central canals bifurcate from the annular constricted region (AC) to the terminal duplicated cord. Each site marked with dotted lines and letters corresponds to the respective parts in Fig. 2.

connection to the syringomyelic cavity, the lobular or outpouching shapes and the increase in number of central canals, are consistent with the characteristics of myelodysplasia [23] and indicate abnormal development. Myelodysplastic changes were often seen in dysraphic states [25]. The myeleterosis at the affected regions in the present case would be due to a failure of secondary neurulation.

In the caudal duplicated terminal segments of the spinal cord, the regional separation of the spinal cord 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 [26]. According to the unified theory [26], in which the split cord malformation originates from an ontogenic error occurring around the time when the primitive neurenteric canal closes, an abnormal fistula formed through the midline embryonic disc with communication between the yolk sac and amnion causes regional 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 [26, 37]. Therefore, the affected site would be rostral to the coccygeal segments that ultimately come to lie opposite the primitive pit. The caudal duplicated terminal segments of the spinal cord and the duplicated filum terminale in the present case could not be explained by the unified theory and would result from an insult to the secondary neural tube derived from the tail bud. The duplication of the terminal spinal cord seems to be closely related to the bifurcated fragmentary central canals from the dysmorphogenetic mechanism. A canalization error might occur in the neural cord derived from the tail bud and induce duplicated cord formation according to the bifurcated fragmentary central canals. Simultaneously, the mesenchyme derived from the tail bud around each bifurcated terminal cord would develop to duplicate the filum terminale. Since central canal forking or duplication was also seen at the region of the conus medullaris, ventriculus terminalis, and filum terminale in normal human children [15, 21], the terminal embryonic neural cord might be more sensitive to primary embryonic misconstruction. The dorsoventral duplication of the terminal spinal cord was associated with bovine segmental spinal dysgenesis with CA [36], which might result from the canalization error, as in the present case. On the other hand, a duplicated filum terminale with a TSC, in which it was not clear whether the terminal region of the caudal spinal cord was duplicated, has been reported in humans [27, 32].

From the above description of the main pathological changes, the malformation in the present case of the spine and spinal cord at the sacrococcygeal regions was diagnosed as a rare anomaly of TSC related to caudal spinal dysraphism, comprising a duplicated terminal cord and filum terminale, which represents the first bovine case definitively confirmed morphologically, to the best of our knowledge.

Embryologically, the tail bud of amniote embryos comprises a mass of apparently undifferentiated mesenchymal cells located at the caudal limit of the embryo, representing Hensen's node and the primitive streak. These cells have the potential to give rise to a variety of different tissues including the posterior or 'secondary' neural tube, tail gut, and somite and their derivatives [16]. At the time of closure of the posterior neuropore, the posterior tip of the neural tube is at the level of the future upper sacral region [3]. In the tail bud, the neural tube is formed by secondary neurulation. The transition from primary to secondary neurulation occurs at the future upper sacral level and would mostly correspond to the affected site in the present case. In the bovine embryo, the closure of the posterior neuropore occurs at approximately 26 intrauterine days (25- somite stage) [17]. From an embryogenetic point of view in the present case, the causal insult would impair the terminal segments derived from the tail bud and would result in TSC with the canalization error.

Fetal infection with Akabane and Schmallenberg viruses often affects the developing spinal cord and induces myelodysplasia and micromyelia, respectively [5, 23]. However, no lesions in this case appeared associated with Akabane and Schmallenberg infections, such as a uniform spectrum of cerebral malformations including hydranencephaly, porencephaly and microcephaly, and localized or generalized arthrogryposis of the axial and appendicular skeleton [2]. Epidemiological evidence for Schmallenberg infection is found only in Europe [13]. In a serological survey of the Akabane virus, the presence of seroconversion in sentinel cattle was not recognized in Kumamoto Prefecture in the fetal period of the subject corresponding to the period between August 2014 and May 2015 [11]. Other viruses such as bovine viral diarrhea virus and some arboviruses, including Aino, Chuzan and Peaton viruses, also infect the developing bovine fetus and induce malformation of the central nervous system and/or the axial and appendicular skeleton [2, 23, 24, 39]. However, the effects on the spinal cord and the caudal vertebral column of intrauterine infection of these viruses remain unknown. Therefore, no objective evidence has been obtained of these viral infections inducing congenital malformation of the central nervous system and/or the axial and appendicular skeleton. A simple genetic cause was eliminated from the present family line in the absence of a common ancestor, and no relationship with the inherited axial spine malformations such as complex vertebral anomaly and brachyspina was confirmed from the pedigree information.

The etiology of 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 maternal diabetes mellitus, insulin, embryonal trauma, severe temperature changes, vitamin deficiency, lithium salts, trypan blue, and retinoic acid which have been implicated as causes of CA [8, 14], have attracted our attention and will represent a future subject of investigation.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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