Introduction

In cases of spina bifida, malformations most frequently occur in the synsacrum. Spina bifida causes partial re- gurgitation of the dorsal lamina of the vertebral arches, whereas the spinous process of a lesion is generally bi- sected laterally. Spina bifida is considered an abnormality of the skeletal system and of the nervous system, as it affects the spinal cord or meninges. This condition occurs in three forms: spina bifida occulta, where the skin covers splits in the vertebrae; spina bifida cystica, in which cysts protrude from the gaps between vertebrae; and open spina bifida, which features complete skin openings. Moreover, spina bifida can cause herniations in which cerebellar tissues form linguiform projections through the great foramen and forward toward the vertebral canal. This phenomenon, also known as the Arnold–Chiari malformation, accompanies many cases of spina bifida. In addition, spina bifida may lead to other complications, including internal hydrocephaly, arthrogryposis, neuromuscular dysplasia, cleft palate, lumbar vertebrae defectus monster, diplcephalus, fused kidneys, uterine horn malformation, scoliosis, phrenic cleft, rumpleness, and undescended testicles.

The present report describes the observation of a calf with spina bifida and giant mass of the lumbar region, which was subjected to gross, histological, and immunohistochemical examinations. A malformed Japanese black calf (estimated weight = 20 kg) was euthanized immediately after birth. A gross evaluation revealed a giant mass (approximately 60 cm × 30 cm × 15 cm) covered by the hair coat in the lumbar region and connected with the hair coat of the trunk. The mass surface was divided by a deep polygonal groove and externally resembled a lobulated kidney. Histology and immunohistochemistry revealed that the giant mass comprised a vessel, bronchiolus lined with cuboidal epithelium, and small alveolus. Bone bleaching revealed various abnormalities, including spina bifida, vertebral fusion, vertebral deformity, vertebral malformation, vertebral scoliosis, and coxal bone malformation. Following a suggestion that the giant lumbar region mass was occupied by lung tissue, this case was considered to involve an asymmetric conjoined duplicitas that resulted in a very rare dichotomous spondylosis malformation.
anized after abnormalities such as an astasia and giant mass were observed.

Macroscopic dissection

A gross macroscopic observation of the calf’s external shape was performed after whole-body perfusion fixation in 10% neutral formalin. The muscles and internal organs were decortized whereas the skeleton was retained in its complete form. Whole-body histological specimens were extracted, and the skull, spinal column, and coxal bone were then evaluated after bone bleaching.

Histology and immunohistochemistry

Whole-body histological specimens were fixed in 10% neutral formalin. The tissues were dehydrated and embedded in paraffin wax. Tissue sections (4–5 μm) were stained routinely with hematoxylin and eosin, azan for collagen, elastica van Gieson (EVG) for elastic fibers, and periodic acid-Schiff (PAS) for acid mucus.

For immunohistochemistry, a peroxide-labeled antibody method and the following primary antibodies were used: anti-cytokeratin AE-1/AE-3 mouse monoclonal serum (Progen GmbH, Heidelberg, Germany), anti-α-smooth muscle actin mouse monoclonal serum (Nichirei, Tokyo, Japan), and anti-thyroid transcription factor-1 (TTF-1) mouse monoclonal serum (Dako Co., Glostrup, Denmark). Peroxidase-conjugated secondary antibodies (anti-mouse IgG; Nichirei) and diaminobenzidine (chromogen; Nichirei) were also used. Sections were counterstained with Mayer’s hematoxylin and mounted under coverslips.

Results

Outward form

A giant mass with approximate measurements of 60 cm × 30 cm × 15 cm was observed in the lumbar region. This giant mass was covered by hair coat and connected with the hair coat of the trunk (Fig. 1A). The head featured a short, bulldog-like nose because the tip curved leftward and superiorly (Fig. 1B). A cleft palate was observed in the uraniscus (Fig. 1C). The vertex was raised, and hydrocephaly was evident. No abnormalities were observed in the appendicular skeleton, despite the lack of a tail.

Autopsy findings

Skin removal revealed that the mass was covered with tunica. The mass surface was divided by a deep, polygonal groove and had the external appearance of a lobulated kidney (Fig. 1D). The cross-section showed a soft and spongy interior, with a white tubular structure running through the middle (Fig. 1E). The spinal cord had branched in the region bisected by a spinous process. Dilation of each side of the lateral ventricle and thinning of the hemispherium were observed in the cerebral cross-section (Fig. 1F). A ventricular septum defect and persistence of patent foramen ovale were observed in the heart.

Bone bleaching findings

No numerical or morphological abnormalities were observed in the cervical vertebrae. The posterior thoracic vertebrae, lumbar vertebrae, sacrum, and coxal bone were abnormally shaped. No abnormalities were observed in the numerical thoracic vertebrae. The vertebral foramen had opened toward the dorsum as a possible consequence of bisection of a spinous process by the sacrum from T12 (Fig. 2A). The foramen curved right-ward from T10 to L2, left-ward L2 and had an overall sigmoidal appearance (Fig. 2B). Remarkable extrusion was observed on the right side of the vertebral body, given the apices of the bisected spinous processes in T8 and T9 and the absence of the left-side vertebral body segmentum in T10. Two transverse and spinous processes were observed to the left of T11. Although the spinous process was also bisected, the upper section of the vertebral body had completely fused. This served to assimilate the upper part of the transverse process to the right side of T10–13 and was deforming. In T13, a bone-like spinous process had extended to the nucleus caudalis. Because lumbar vertebral fusion might have complicated the spinous process, it was difficult to count these processes from the dorsum; therefore, six vertebral bodies were identified via counting from the ventral surface. In L2 and L3, the intervertebral foramina had disappeared, allowing the right-hand side vertebral arch to fuse. In L3, two spinous and transverse processes were observed at the left-side of one vertebral body, and the disappearance of the intervertebral foramina allowed complete fusion of the spinous process. Marked malformation was observed in the sacrum, and the posterior, right-side transverse and spinous processes had disappeared completely. Coccygeal vertebrae were not observed, and the coxal bone boundary was clear because the pars symphysica of the iliac bone, pubic bone, and ischium had not ossified completely. The pubic and iliac bones had not connected, thus allowing observation of the malformed acetabular fossa.

Light-microscopy findings

The giant mass comprised a vessel, bronchiolus lined with cuboidal epithelium, and a small alveolus; in other words, the histological structure was similar to that of lung tissue (Fig. 3A). The bronchiolus, which connected to the small alveolus, gradually thinned and branched asymmetrically, with losses of epithelial cells in several places. The bronchiolar epithelial cells exhibited positive cytoplasmic PAS staining, and the interalveolar septum was surrounded by elastic and collagen fibers, as demonstrated by EVG and Azan staining, respectively (Fig. 3B).
Fig. 1. Outward form and autopsy findings. (A) Giant mass in the lumbar region. (B) Bulldog-like head. (C) Cleft palate. (D) Appearance of giant mass after skin removal. The external appearance is similar to a lobulated kidney. (E) Cross-section of the giant mass. (F) Cerebral cross-section, showing dilation of the ventriculus lateralis cerebri and thinning of the hemispherium.
**Immunohistochemistry findings**

In the mass tissue, bronchiolar and alveolar cells exhibited positive cytoplasmic staining with an antibody specific for cytokeratin AE-1/AE-3 (Fig. 4A) and frequent positive nuclear TTF-1 antibody staining (Fig. 4B). Vessels of various sizes and many α-smooth muscle actin antibody-positive capillaries were observed in areas surrounding the bronchioles and alveolar septum.

**Discussion**

The giant mass observed in the lumbar region of a calf comprised blood vessels, bronchioli (terminal and respiratory), alveolar ducts, alveolar sacs, and alveolus. However, a bronchus was not observed. Furthermore, fibrosis caused by collagen fiber proliferation had occurred around the alveolus. These findings suggest that the giant mass comprised lung tissue. The mass was occupied by an alveolar duct and alveolus, which are characteristic...
Giant lumbar mass in a newborn calf

of normal lung tissue, surrounding a small number of bronchioli, including terminal and respiratory bronchioli. Moreover, smooth muscle and connective tissues were detected around the bronchiolus, terminal bronchiole, and a thick vessel. Many capillaries can be observed in the alveolar septum\textsuperscript{9}. During lung development, the primordial respiratory organ generated at the abdominal midline gradually forms a long cul-de-sac or primary lung bud. Next, the primary lung bud elongates to form a trunk bronchus, and evagination of the tips creates a secondary lung bud. Extension and branching of the lung bud forms the lung, which gradually moves to the caudalis\textsuperscript{7}). Lung maturation is divided into four periods: pseudoglandular, canalicular, saccular, and alveolar. The bronchus and terminal bronchiole form during the pseudoglandular period, and the respiratory bronchiole and alveolar duct form during the canalicular period. A primitive alveolus forms during the saccular period, and pulmonary alveoli mature during the alveolar period\textsuperscript{7}). Therefore, during development, the lung forms from the bronchus toward the pulmonary alveoli. However, the lung tissue observed within the mass in this case did not contain a bronchus but was occupied by the bronchiole. These findings suggest that the lung within the mass was an imperfect, rather than developing, lung.

The gross features of this case suggest asymmetric conjoined duplicitas. A duplicitas, or double individual, generates an identical twin. This phenomenon occurs because the whole body or a part has been duplicated during an abnormal embryonic growth process of uniovular twins. A duplicitas can be classified as follows: a single normal child, acardius, or asymmetric separated conjoined twin of a malformed child; systemic or partially symmetric conjoined duplicitas that combines two individuals; and asymmetric conjoined duplicitas, in which a parasite attaches to the normal autosite\textsuperscript{8−10}). Duplicitas occurs relatively frequently in calves, with an approximate incidence of one per 100,000 animals\textsuperscript{11, 12}). The etiology of duplicitas remains largely unclear\textsuperscript{13}).

The giant mass observed in this case might be associated with dichotomous spondylosis, an extremely rare condition that has only been associated with a mass in a calf in one previous report. In that case, the mass was not reported to comprise lung tissue. Only one other example of a Japanese black calf with a 23 cm × 16 cm mass in the lumbar region has been reported to date. A papillary- or mammary-like structure accompanied that mass, which contained the gut and spleen of a parasite. The vertebra in the mass region was also cleaved. These findings led to the designation of the earlier Japanese black calf as an asymmetric conjoined duplicitas that enclosed a parasite subcutaneously within the dorsal region. Regurgitation of a dorsum vertebral arch is considered the pathogenic event underlying the occurrence of spina bifida during the neurulation period. Because the neural canal, notochord, and paraxial mesoderm are closely related during development, a single initial abnormality is thought to influence development of the entire side\textsuperscript{14}), leading to various secondary abnormalities such as vertebral fusion, deformity, and malformation; vertebral scoliosis; coxal bone malformation; cleft palate; rumplelessness; spinal cord bifurcation; and internal hydrocephaly. These abnormalities of the central nervous system were observed as a consequence of spina bifida in the present case.

Fig. 4. Immunohistochemistry for the giant mass. (A) Bronchiolar and alveolar cells exhibited cytoplasmic cytokeratin AE-1/AE-3 positivity (bar = 100 μm). (B) Alveolar cell nuclei were often positive for TTF-1 (bar = 50 μm).
In conclusion, the findings of this case suggest that the giant mass observed in the lumbar region contained lung tissue. This malformed calf was therefore considered an asymmetric conjoined duplicitas, and the case was considered a very rare example of dichotomous spondylosis.

References