NOTE *Internal Medicine*

Thymic Lymphosarcoma with Brain Involvement in a Holstein Heifer

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ABSTRACT. An 8-month-old Holstein heifer with cervical enlargement was suspected of thymic lymphosarcoma given clinical signs of depression, tendency to lie down, cervical mass, jugular vein distension, conjunctival hyperemia, and ruminal tympany. Unilateral Horner's syndrome was also observed. Increased serum total lactate dehydrogenase (LDH), LDH isozyme (LDH-2, and LDH-3) and serum thymidine kinase activity were observed. The findings of fine needle aspiration cytology of the cervical mass revealed large lymphoblasts with mitoses present. These findings strongly suggested the diagnosis of lymphosarcoma. Necropsy revealed a large mass in the cervical thymic region, which compressed the esophagus and trachea. Cranial masses in the frontal sinus and multiple extradural sites throughout the cranial vault were also recorded. Histopathological examination confirmed the diagnosis of thymic lymphosarcoma and demonstrated the brain involvement of neoplastic lymphoid cells in the cerebrum. This is a rare clinical case of thymic lymphosarcoma accompanied by brain metastasis in a Holstein heifer.

KEY WORDS: brain involvement, cattle, thymic lymphosarcoma.

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Bovine lymphosarcoma is one of the most common types of neoplasm affecting dairy cattle. In general, it can be divided into 2 types: enzootic bovine lymphosarcoma (EBL) caused by bovine leukemia virus (BLV) and sporadic bovine lymphosarcoma (SBL) [3]. SBL has no known cause, does not appear to be associated with BLV, and has a much lower prevalence [3]. SBL can be further subdivided into three forms: calf, juvenile/thymic and skin forms [19]. The thymic form of lymphosarcoma usually is observed in 6- to 24-month-old cattle, and is characterized by a large, firm swelling of the ventral neck region [4, 6]. Progressive thymic enlargement occurs in all cases and is clinically apparent when cervical enlargement develops. Cattle with thymic lymphosarcoma often have clinical signs of brisket and submandibular edema, and jugular vein distension [7, 8]. The main clinical signs resulting from thymic lymphosarcoma are either bloat or dyspnea. Macroscopic neoplastic lesions are distributed across lymph nodes, bone marrow, liver, spleen, kidneys, lungs, and thymus [13]. Brain and spinal cord are usually not directly affected in this form of SBL [1, 2, 4, 7, 10, 13, 16]. Here, we present a rare clinical case of thymic lymphosarcoma accompanied by brain involvement in a Holstein heifer.

An 8-month-old Holstein heifer with cervical enlargement was initially examined by a local veterinarian. At examination, the heifer had a body temperature of 38.6°C and heart rate of 70 bpm, and showed bloat and anorexia. Despite treatment with antibiotics and dexamethasone, the heifer's general condition did not improve. On the third day of illness, the heifer was taken to the Veterinary Teaching Hospital, Obihiro University of Agriculture and Veterinary Medicine. Upon admission, rectal temperature was 39.0°C, heart rate 90 bpm, and respiratory rate 26 breaths/min. Physical examination revealed a cervical mass measuring 30 × 20 × 20 cm (Fig.1), jugular vein distension, conjunctival hyperemia, and ruminal tympany. Tympany was caused by free gas, which was easily released using a nasal-gastric tube. Peripheral lymph node enlargement was not detected. The heifer also showed signs of depression and had a tendency to lie down on its side. Drooping of the upper eyelid, miosis and enophthalmos were observed on the right side of the face. Hematologic examination performed using hematological analyzer, showed microcytic and hypochromic red blood cells (RBC, $8.33 \times 10^6/\mu l$; hemoglobin, 9.6 g/d*l*; hematocrit, 29.1%; mean corpuscular volume, 34.9 fl; and mean corpuscular hemoglobin, 11.5 *p*g) and leukocytosis (WBC, 14,600/μl; neutrophils, 2,920/μl; lymphocytes, 11,534/μl; and monocytes: 146/μl). More than 80% of lymphocytes were morphologically atypical with wide cytoplasm, fine nuclear chromatin and nucleoli. The platelet counts (360,000/μl) were within the reference range. Serum creatinine, urea nitrogen, total protein, albumin, and serum calcium levels were normal. Antibodies against BLV were not detected with agar-gel immunodiffusion with commercial antigen (Kitasato Institute Research

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Fig. 1. An 8-month-old Holstein heifer with cervical enlargement (arrows).

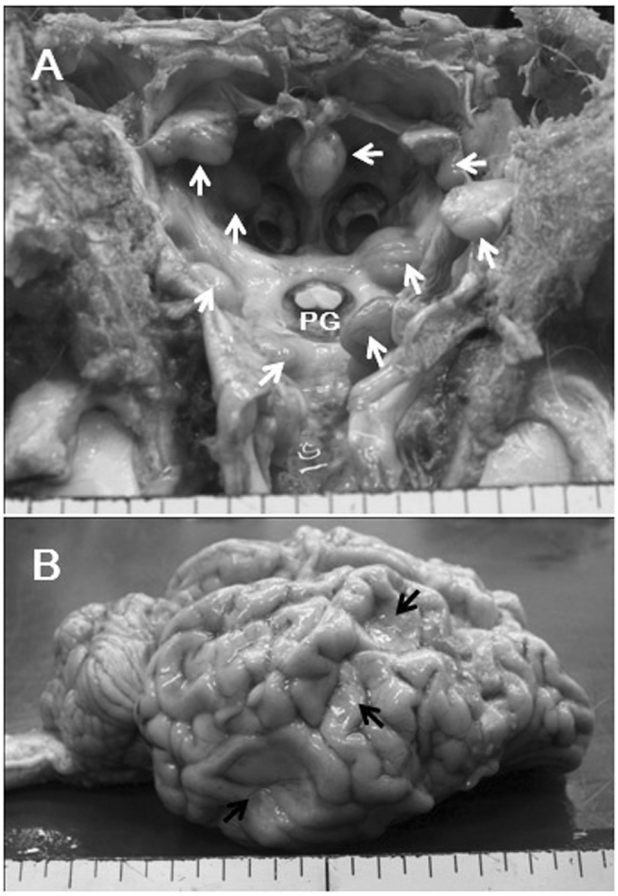

Fig. 2. Postmortem specimens of frontal sinus and cerebral hemishere. (A) Several small masses (indicated by white arrows) were in the frontal sinus, and multiple extradural sites throughout the cranial vault. PG: pituitary gland. (B) Cerebral hemisphere shows compression by neoplastic masses (black arrows).

Center for Biologicals, Saitama, Japan). Serum biochemical analysis showed elevated aspartate aminotransferase (AST: 204 IU/*l*) and lactate dehydrogenase (LDH: 2,740 IU/*l*) activities. LDH isozyme analysis showed elevated activities for LDH-2 (994 IU/*l*) and LDH-3 (264 IU/*l*). Total LDH, LDH-2 and LDH-3 activities have been known as lymphosarcoma markers [12], although their specificities are insufficient to confirm a diagnosis of lymphosarcoma. Thymidine kinase (TK) activity, which is thought to be a biomarker of bovine leukemia with higher specificity [18], was also measured from serum samples using a commercial radioenzyme TK-assay kit (Kishimoto Clinical Laboratory, Inc., Obihiro, Japan). TK activity was increased (132.6 IU/*l*). The findings of fine needle aspiration cytology of the cervical mass revealed large lymphoblasts with mitoses present. These clinicopathological findings strongly suggested that the present case was SBL, a thymic lymphosarcoma.

The heifer was euthanized, and necropsy was performed on day 4 of illness. Gross examination revealed a solid mass (30 × 20 × 20 cm) in the cervical thymic region around the trachea, and it compressed the esophagus and trachea. Several small masses ranged from 1 to 8 cm in diameter were found in the thoracic thymus, pleura, liver, uterus, ureters, and perirenal tissue. Abdominal lymph nodes also showed slight enlargement. In addition, several small masses ranged from 0.5 to 1.5 cm in diameter were found in the mucosa of frontal sinus, and multiple extradural sites throughout the cranial vault, including cerebral dura mater, surrounding the pituitary gland tissue, and exterior dura mater of the medulla oblongata (Fig.2A). The cerebral hemisphere showed extensive compression due to neoplastic masses (Fig.2B). Histopathological examination revealed that the cervical mass was composed entirely of neoplastic lymphocytes with irregular nucleus and different in size. Mitotic cells were often observed. Similar neoplastic cells were also observed in small masses in other organs. Neoplastic cells were also in leptomeninges and perivascular spaces of the cerebrum (Fig. 3). Immunohistochemical examination showed tumor cells positive for CD3 and negative for BLA-36 antibodies. Mild ischemic changes were observed in nerve cells of cerebral compression sites. The diagnosis of thymic lymphosarcoma was confirmed by these pathological findings.

Involvement of the central nervous system (CNS) is a common clinical observation in EBL [5]. However, the brain and spinal cord are usually not directly affected by the thymic form of lymphosarcoma [1, 2, 4, 7, 10, 13, 16]. One exception was a clinical case of thymic lymphosarcoma with metastases causing spinal cord compression and pelvic limb paresis in a heifer [11]. To the best of our knowledge, macroscopic brain involvement has not been reported in thymic lymphosarcoma. In the present case, several cranial masses were recorded (i.e., frontal sinus, cerebral dura mater, sur-

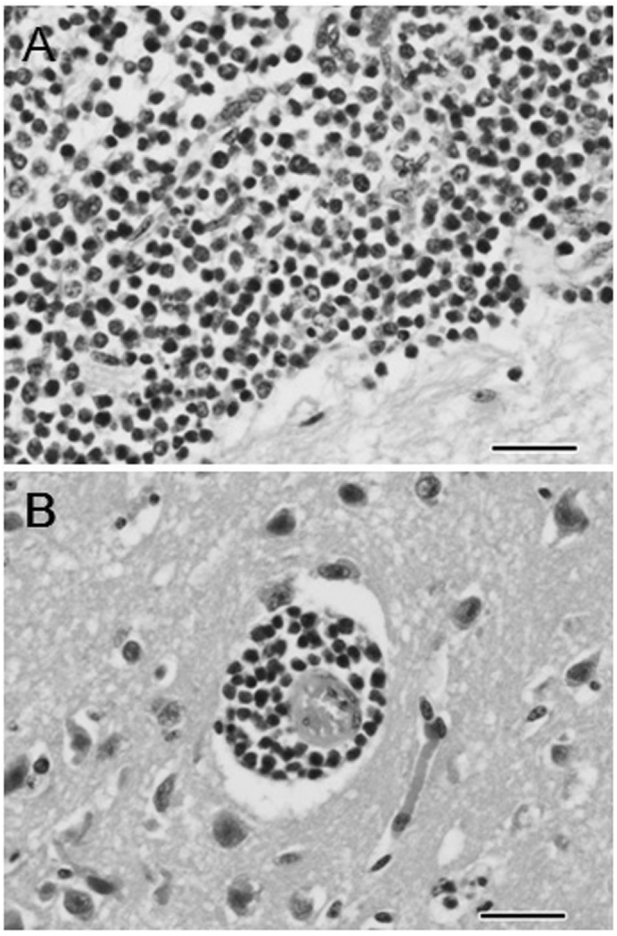

Fig. 3. Histopathology of cerebrum. Neoplastic lymphoid cells were in leptomeninges (A) and perivascular spaces (B) of the cerebrum. Haematoxylin and eosin stain. Bar=25 μm.

rounding pituitary gland tissue, and exterior dura mater of the medulla oblongata). Clinical signs of depression and a tendency to lie down were most likely caused by ischemic changes of nerve cells in the cerebral compression. Horner's syndrome results from interruption of ocular sympathetic pathways, from the midbrain close to pituitary gland through the spinal cord down to T1-T3 spinal segments, up the vagosympathetic trunk, and cranial cervical ganglion next to the tympanic bulla [14, 15, 17]. Specific causes include traumatic lesions to the basisphenoid region, cervical trauma, abscesses, tumors, or space-occupying lesions in the anterior aspect of the thorax. In cattle, it has been associated with abscesses and cranial tumors, including adenocarcinoma and squamous cell carcinoma [6, 9, 14]. In the present case, ocular sympathic pathway close to pituitary gland might be affected by compression of extradural masses found in the cranial vault. Another possibility is unilateral damage of ocular sympathic pathway by cervical tumor caused unilateral Horner's syndrome; however, the real cause was not clarified.

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