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SHORT COMMUNICATION Clinical Case Report

A clinical case of enzootic bovine leukosis diagnosed by malignant lymphoma cells detected in urinary sediment

Kana Sakaguchi[1)], Manabu Ougitani[2)], Masaki Maezawa[1,3)], Yusuke Tanaka[1)], Ken-ichi Watanabe[1)], Noriyuki Horiuchi[1,3)], Yoshiyasu Kobayashi[1,3)] and Hisashi Inokuma[1,3)]

[1)] Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[2)] Japan Agricultural Cooperatives-Shihoro, Shihoro, Hokkaido 080-1200, Japan
[3)] United Graduate School of Veterinary Science, Gifu University, Gifu 501-1193, Japan

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Abstract

A Holstein cow presented with decreased milk production. Rectal palpation revealed masses in the pelvic cavity without enlargement of superficial lymph nodes. The cow maintained a continuing urination posture after urination and yellow cloudy urine drops were observed. Cytology of urine sediment revealed large atypical lymphoid cells suggestive of lymphoma. Hematology examination showed lymphocytosis with atypical lymphocytes. Increased activities of serum lactate dehydrogenase and thymidine kinase were also suggestive of lymphoma. PCR for bovine leukemia virus was positive. B-cell clonality PCR to define the rearrangement of immunoglobulin heavy chain revealed monoclonal growth of B-cells. These findings led to a diagnosis of enzootic bovine leukosis (EBL). Examination of urinary sediment can be diagnostic for EBL with lesions in the urinary system.

Key Words: clinical diagnosis, enzootic bovine leukosis, urinary sediment

Bovine leukosis is a common neoplastic disease in cattle. Enzootic bovine leukosis (EBL) is caused by bovine leukemia virus (BLV). Enlargement of superficial lymph nodes (SLN) is an important clinical finding that can lead to a suspicion of EBL. Examination of BLV infection and cytology of enlarged SLN obtained by fine-needle aspiration (FNA) are used for definitive diagnosis of EBL[1, 8)]. In cases without enlargement of SLN, however, EBL is often unsuspected and diagnosis is difficult. In the present report, a clinical case of EBL without enlargement of SLN was clinically diagnosed by the detection of malignant lymphoma cells in urinary sediment.

A 3-year, 10-month-old Holstein dairy cow presented with decreased milk production 4 months after giving birth and was presented to a local veterinarian. On day 2, the cow exhibited anorexia, continuing urination posture, and stopped milking. Enlargement of SLN was not observed, but a pelvic mass (20 cm) was detected by rectal palpation. Despite treatment with an

* Corresponding author: Hisashi Inokuma, DVM, PhD, Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080-8555, Japan
Phone: +81-155-49-5370. E-mail: inokuma@obihiro.ac.jp
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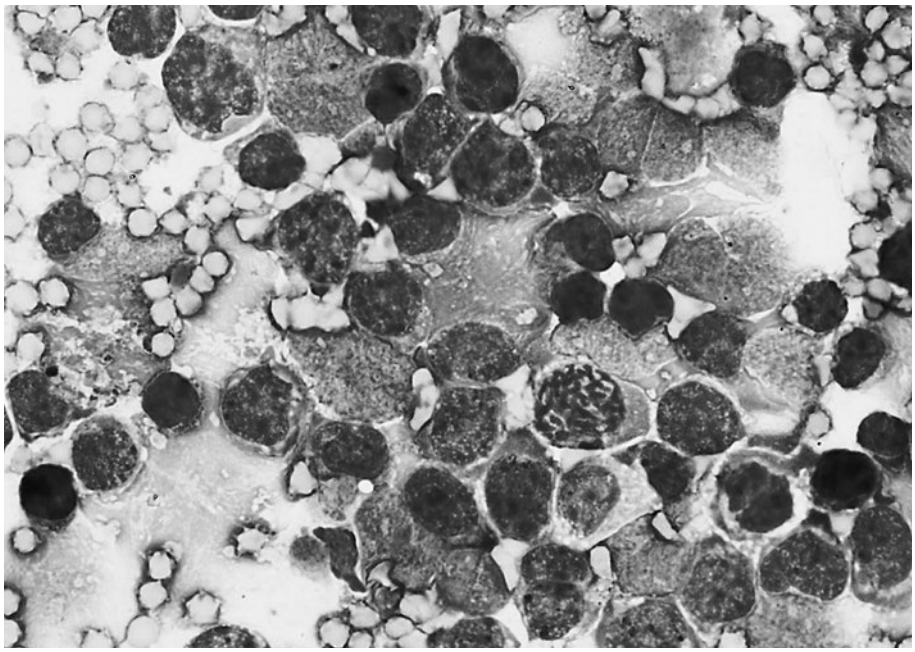

Fig. 1. Large malignant lymphocytes with marked atypia and fine nucleoli were observed in urinary sediment. Wright-Giemsa stain.

intravenous drip containing calcium gluconate and glucose, the general condition of the cow did not improve. The cow was transferred to the Animal Teaching Hospital at the Obihiro University of Agriculture and Veterinary Medicine for further investigation on day 23. On initial physical examination, the cow had a high rectal temperature (40.0 °C), tachycardia (100 beats/min), normal respiration rate (36 breaths/min), emaciation, anorexia, diarrhea, enophthalmos, and a prolonged skin tent test. Enlargement of SLN was not observed. Rectal palpation revealed two masses (20 cm) in the pelvic cavity under the right ilium. Ultrasound examination using a transrectal probe revealed the masses in the pelvic cavity to have an echo pattern containing hyperechoic areas, denying abscess or cyst. The uterus could not be felt by rectal palpation. Colposcopy revealed the vagina to contain white purulent fluid and a congested vaginal wall. After urination, the cow maintained a continuing urination posture and yellow cloudy urine drops were observed (Fig. S1). These findings led to a suspicion of a urinary tract abnormality. Cytology of urine sediment revealed many large lymphoid cells with marked atypia and fine nucleoli, which suggested lymphosarcoma (Fig. 1).

Hematological examination showed lymphocytosis (13,783/μl); 26 % of lymphocytes were atypical with wide cytoplasm, fine nuclear chromatin, and nucleoli. Serum biochemical analysis revealed increased levels of aspartate aminotransferase (AST: 194 U/l), alkaline phosphatase (ALP: 241 U/l), creatinine phosphokinase (CPK: 2,254 U/l), blood urea nitrogen (BUN: 115.9 mg/dl), creatinine (CRE: 4.9 mg/dl), total lactate dehydrogenase (LDH: 3,480 U/l), and thymidine kinase (TK: 67.8 U/l). LDH isozyme analysis showed elevated activities of LDH-2 (1,114 U/l) and LDH-3 (452 U/l).

DNA samples were obtained from peripheral blood using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) to test for BLV infection and B-cell clonality. For the genetic diagnosis of BLV infection, nested polymerase chain reaction (PCR) for the BLV 5' long terminal repeat (LTR) was performed with the following primers: F1/R2 (F1: 5'-GTG-CTC-AGC-TCT-CGG-TCC-TGA-GC-3',

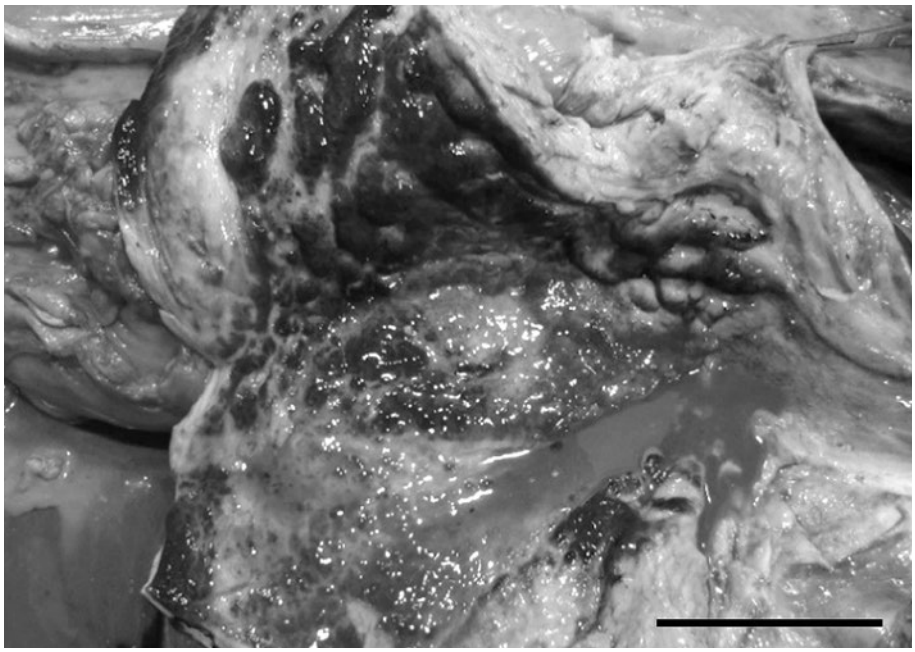

Fig. 2. Bladder membrane (ventral view) was swollen and the cutting surface was firm, homogenous, and white to grey. Bar=10 cm.

R2: 5'-AAA-CAA-ACG-CGG-GTG-CAA- GCC-AG-3') for outer PCR, and F2/R1 (F2: 5'-TCT-CTT-GCT-CCC-GAG-ACC-3', R1: 5'-AGG-AGG-CAA-AGG-AGA-GAG-T-3') for inner PCR[4]. These primers were designed based on the sequence of the LTR of BLV. PCR conditions were the same as previously reported[4]. The amplicon was confirmed by electrophoresis on a 2 % agarose gel. A single band suggested the DNA sample was BLV positive. B-cell clonality of peripheral blood was also examined using PCR to amplify the variable region of the immunoglobulin heavy chain gene. DNA extracted from peripheral blood was used as a template for PCR performed with HotStartTaq DNA Polymerase (QIAGEN). PCR was performed with the following primers: BoVHF1: 5'-AGC-CCT-GAA-ATC-CCG-GCT-CA-3', BoVHR1: 5'-TCC- AGG-AGT-CCT-TGG-CCC-CA-3'. PCR conditions were the same as previously reported[4]. The amplicon was electrophoresed on a 3 % agarose gel, and a single band suggested B-cell monoclonality (Fig. S2). The nucleotide sequence of the amplicon was determined using the BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) and ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.). The determined sequence was compared to registered sequences using BLAST (National Center for Biotechnology Information) and found to be most closely related to '*Bos taurus* immunoglobulin rearranged heavy chain variable region mRNA (U49777.1).'

The cow was euthanized under anesthesia on day 26 for necropsy. In the pelvic cavity, three masses were found on the right and left sides of the uterine body and on the broad ligament of the uterus (Fig. S3). The cut surfaces were firm, homogenous, and white to grey. The uterus and surrounding connective tissues were swollen. Membranes of the ureter, bladder, urethra, and abomasum were also swollen and their cutting surfaces were firm, homogenous, and white to grey as well (Fig. 2). Obvious enlargement of lymph nodes in the thoracic or peritoneal cavity was not observed.

Histopathologically, neoplastic lymphoid cells diffusely proliferated in the ureter, bladder,

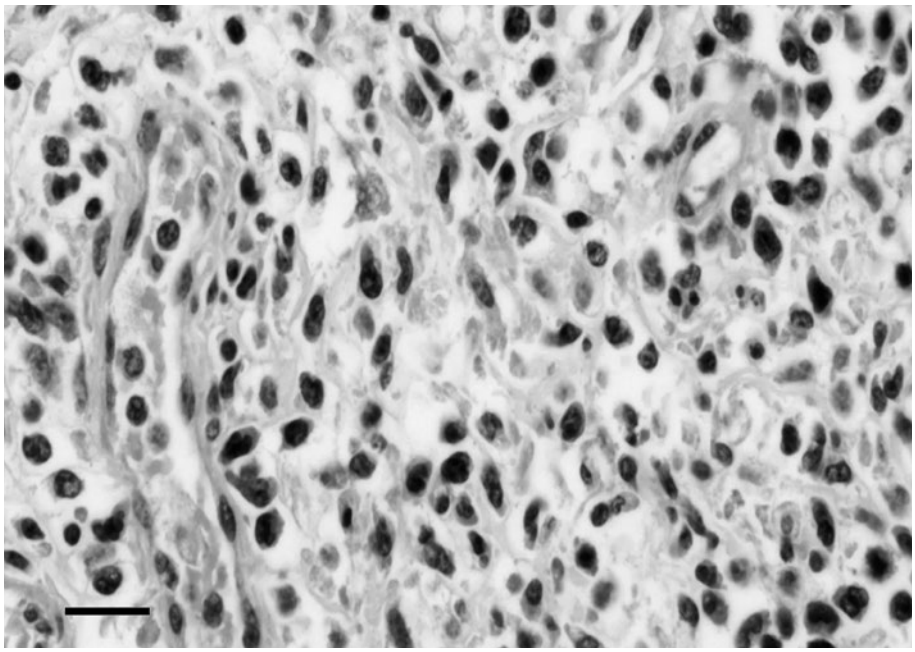

Fig. 3. Histopathology of bladder membrane. The mucosal layer of the bladder membrane infiltrated with numerous neoplastic lymphocytes was not lined with a layer of transitional epithelial cells. HE stain, ×400, Bar=20 μm.

uterus, and abomasum. These neoplastic cells were large and round with a small amount of cytoplasm, and showed irregular thickening of nuclear membranes, crafted nuclei with nuclear atypia, and granular-patterned chromatin. Unswollen SLN, including cervical and axillary lymph nodes, did not have neoplastic cells. The mucosal layer of the bladder membrane infiltrated with numerous neoplastic lymphocytes was not lined with a layer of transitional epithelial cells (Fig. 3). Immunohistochemistry was conducted on the bladder masses and ureter using antibodies to BLA36 (marker of B-cell lymphocytes; BioGenex Laboratories, Fremont, CA, USA) and CD3 (marker of T-cells; Dako, Tokyo, Japan). The majority of neoplastic cells were positive for BLA36 and negative for CD3 (Fig. S4). Based on these results, the neoplasm in the urinary system was diagnosed as B-cell lymphoma.

Activities of total LDH, LDH-2, LDH-3, and TK have been used as bio- markers for diagnosing EBL[3,6,7]. The present case showed higher activities of these enzymes, supporting the diagnosis of EBL. B-cell clonality PCR can be used to diagnose lymphoma by assessing immunoglobulin heavy chain rearrangement. The present case showed a band that was suggestive of malignant monoclonal B-cell lymphocytosis.

Pathological examinations led to a diagnosis of EBL. Specifically, EBL was suspected due to the finding of lymphocytosis with atypical lymphocytes and pelvic masses. SLN cytology by FNA is not the optimal method for definitive diagnosis, particularly since 20 %of EBL cases reportedly do not present with enlarged SLN[2]. In the present case, malignant lymphoma cells detected in urinary sediment were key to reaching a clinical diagnosis. Although B-cell clonal analysis and immunohistochemistry was not performed for the urinary sediment, the finding of B-cell lymphoma in the urinary system strongly suggest that the urinary sediment included malignant B-cell lymphoma cells. In 59 % of bovine lymphoma cases, infiltration of the kidney and ureter was observed and 13 % of such cases presented with urinary abnormalities[5]. However, no study has reported on the usefulness of urinary sediment for diagnosing EBL. Obtaining

and examining urinary sediment is cost-effective, quick, and easy. For cases such as ours which do not present with enlarged SLN, neoplastic cells could be proliferating in the membranes of urinary organs. In such cases, examining urinary sediment can contribute to a clinical diagnosis.

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