NOTE *Internal Medicine*

Calf Form Bovine Leukosis with Lameness in a Holstein Heifer

Mohammad Monir TAWFEEQ[1, 2], Saori MIURA[1], Yuuki NAKANISHI[3], Kazuya SUGIMOTO[3], Yoshiyasu KOBAYASHI[3], Hidefumi FURUOKA[3] and Hisashi INOKUMA[1]*

[1]*Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido, 080–8555, Japan*
[2]*Faculty of Veterinary Science, Kabul University, Jamal Mina Kabul, Afghanistan*
[3]*Department of Basic Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido, 080–8555, Japan*

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ABSTRACT. A 12-month-old Holstein heifer with anorexia, lameness, and enlargement of peripheral lymph nodes was suspected of having bovine leukosis. Although lymphocytosis was not observed, cytology of fine needle aspirate from a superficial cervical node, and increased serum lactate dehydrogenase and thymidine kinase activities, strongly suggested lymphosarcoma. Increased numbers of mononuclear cells as well as mitotic cells were observed in synovial fluid collected from swollen joints. Pathological examination confirmed B-cell calf form bovine leukosis and joint swelling related to neoplastic cell infiltration. Both interleukin-2 receptor and thymidine kinase 1 genes were highly expressed in cells from superficial cervical lymph node aspirate.
KEY WORDS: calf form bovine leukosis, gene expression, joint infiltration.

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Bovine leukosis is one of the most common neoplasms detected in dairy cattle and is generally divided into 2 types: enzootic bovine leukosis caused by bovine leukemia virus (BLV) and sporadic bovine leukosis, which has not known cause and does not appear to have any association with BLV [1]. The sporadic form of bovine leukosis can be further subdivided into calf, juvenile/thymic, and cutaneous forms [3, 15]. Thymic and calf forms of leukosis can affect calves and heifers from birth up to 18 months of age [15]. Younger cattle are generally affected by the B-cell calf form [15, 17]. The most obvious clinical sign of the calf form is diffuse lymphadenopathy that results in obvious and palpable enlargement of peripheral lymph nodes [3]. Tumor cells often infiltrate many organs, including the thymus, lymph nodes, liver, spleen, lung, bone marrow, mammary gland, salivary gland, gall bladder, adrenal gland, and pancreas [11]. Ataxia is another common clinical sign of the calf form, because tumor cells often invade the central nervous system [11, 14]. However, tumor cell infiltration into the musculoskeletal system, such as muscles and joints, is not common in the calf form. In this report, we describe a clinical case of calf form bovine leukosis with lameness and joint swelling related to neoplastic cell infiltration in a Holstein heifer. Additionally, we evaluated enzymatic activity and gene expression related to leukosis as biomarker candidates for the onset of bovine leukosis.

A 12-month-old Holstein heifer presented with anorexia and lameness. At initial examination, the heifer had a body temperature of 38.8°C and heart rate of 108 beats/min (bpm). Diffuse lymphadenopathy was noted. Despite treatment with antibiotics and dexamethasone, the general condition of the heifer did not improve. The heifer was taken to the Veterinary Teaching Hospital at Obihiro University of Agriculture and Veterinary Medicine on day 9. Upon admission, emaciation, depression, lameness, abdominal posture − standing under, and swollen carpal and tarsal joints were noted (Fig. 1). Rectal temperature was 39.9°C, heart rate was 120 bpm, and respiratory rate was 56 breaths/min. Swelling of peripheral lymph nodes, including superficial cervical, subiliac, parotid, mandibular, and mammary lymph nodes, was observed.

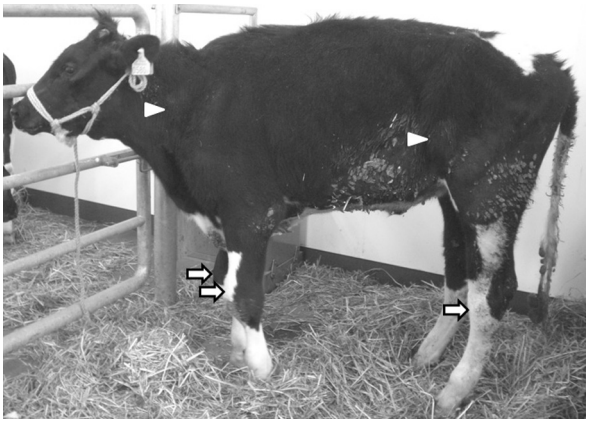

Fig. 1. Emaciation, depression, lameness, standing under, swollen carpal and tarsal joints (arrows), and enlargement of peripheral lymph nodes (arrowheads) were recorded on day 9.

*CORRESPONDENCE TO: INOKUMA, H., Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, Japan.
e-mail: inokuma@obihiro.ac.jp

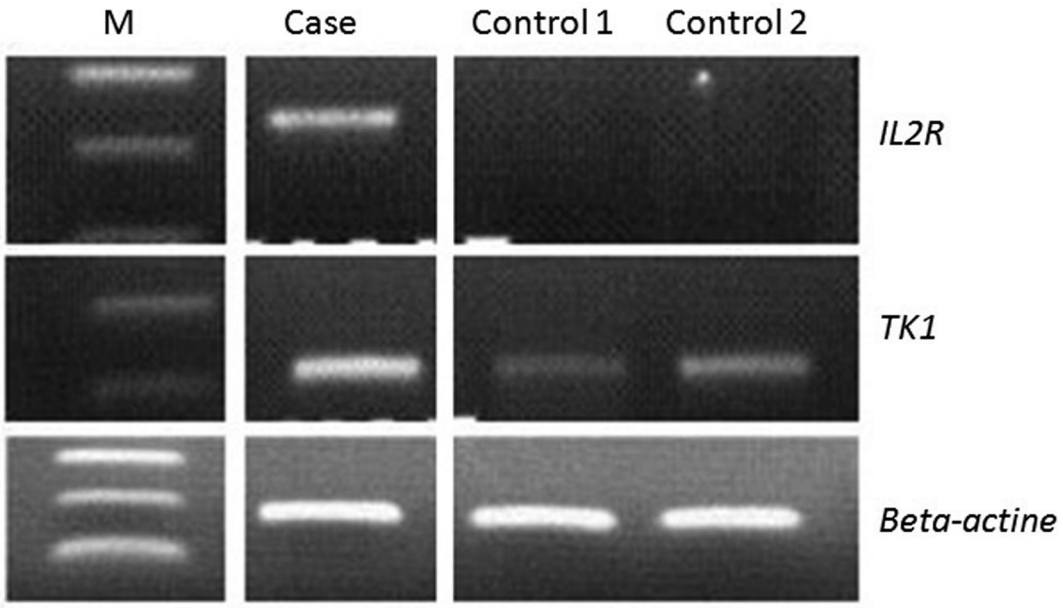

Fig. 2. RT-PCR analysis of *IL2R, TK1*, and beta actin genes in lymph node tissue of the present case and two control animals. M: DNA ladder.

Hematological examination did not reveal any abnormalities such as anemia or lymphocytosis (red blood cell count: $10.56 \times 10^6/\mu l$; hemoglobin concentration: 12.5 g/d*l*; hematocrit: 37.0%; white blood cell count: 10,700/μl(neutrophils: 5,922/μl; lymphocytes: 4,708/μl)). Microscopic examination of peripheral lymphocytes was normal. Serum biochemical analysis showed low total cholesterol (81 mg/d*l*) and increased lactate dehydrogenase activity (LDH: 1,700 IU/*l*). LDH isozyme analysis showed slightly elevated activities for LDH-2 (583 IU/*l*) and LDH-3 (264 IU/*l*) compared to normal [5]. A serum thymidine kinase (TK) activity assay performed using a commercial radioenzyme TK-assay kit with a ^{125}I-iododeoxyuridine tracer (Kishimoto Clinical Laboratory, Inc., Obihiro, Japan) showed increased serum TK activity of 6.8 IU/*l*. An agar gel immunodiffusion assay for antibodies against bovine leukemia virus was negative (Kitasato Institute Research Center for Biologicals, Saitama, Japan). Cytology of fine needle aspirate from a superficial cervical lymph node revealed several large lymphoid cells with marked atypia and mitotic cells, suggesting the diagnosis of lymphosarcoma. We performed arthrocentesis of the swollen carpal and stifle joints using a 23-gauge needle. Synovial fluid collected from each joint was yellow and cloudy, with 20 to 50 cells observed per high dry (40X) field in a stained smear with hemacolor® (Merck Chemicals, Darmstadt, Germany). Most cells were mononuclear, and mitotic cells were often observed.

We examined messenger RNA (mRNA) expression of interleukin-2 receptor (*IL2R)* and thymidine kinase 1 (*TK1)* by reverse transcription-polymerase chain reaction (RT-PCR) using the following primer sets: IL2R-F:5′- acg-cca-tgt-tca-agg-tct-tc −3′ and IL2R-R: 5′-gtt-ctg-cgc-atc-tgt-gtg-tt −3′, and TK1-F: 5′-cca-ggt-tgc-cca-gta-caa-gt-3′and TK1-R: 5′-tct-cgc-aga-act-cca-caa-tg-3.’ We examined beta-actin expression as an internal control using the primer set Actb-F: 5′-ctt-tcc-agc-ctt-cct-tcc-t-3′ and Actb-R: 5′-ggg-cag-tga-tct-ctt-tct-g-3′. We performed RT-PCR on fine needle aspirate from the swollen superficial cervical lymph nodes that contained tumor cells of the case animal and from the superficial cervical lymph nodes of two healthy heifers as controls. We extracted total RNA using the RNeasy Mini Kit (QIAGEN, Germantown, MD, U.S.A.) according to the

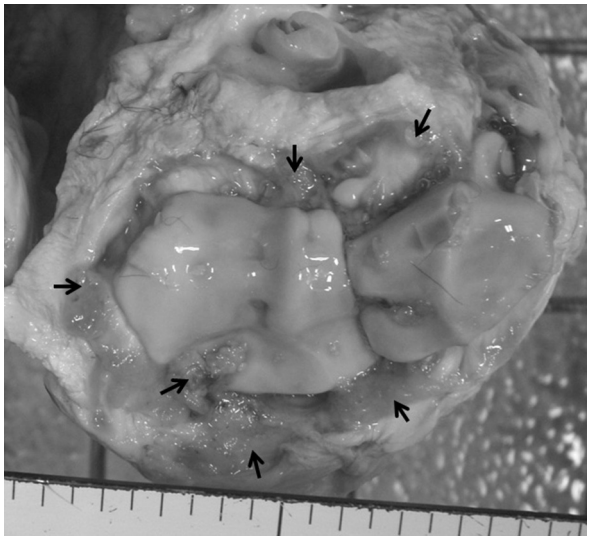

Fig. 3. Yellowish brown gelatinous material similar to synovial villous hypertrophy (arrows) observed periarticularly in the carpal joint.

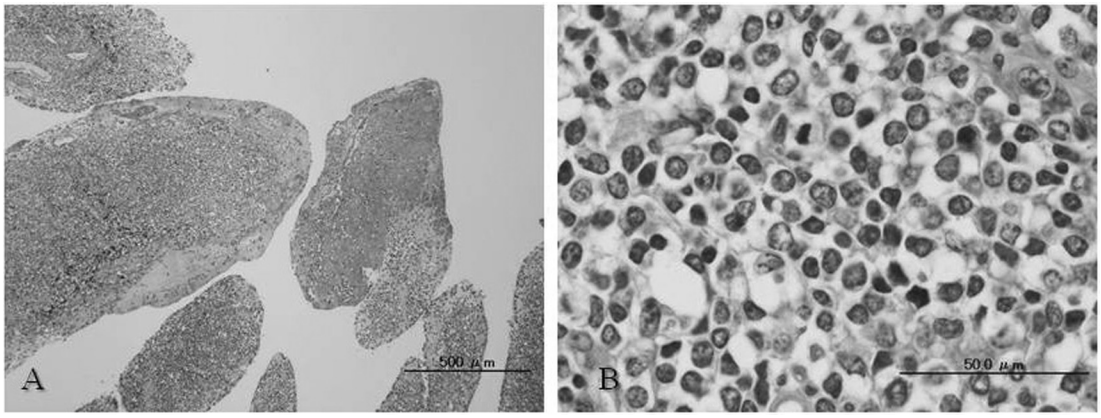

Fig. 4. Histopathological abnormality of the synovial membrane of the carpal joint. (A) The synovial membrane was infiltrated with neoplastic cells and showed papillary projections (hematoxylin and eosin, ×100). (B) The neoplastic cells were large lymphoid cells with obvious atypia (hematoxylin and eosin, × 400).

manufacturer's instructions and synthesized cDNA using 2 μg total RNA and the SuperScript™ III first-strand synthesis system (Invitrogen, Carlsbad, CA, U.S.A.). Both *IL2R* and *TK1* were highly expressed in tumor tissue compared to control animals (Fig. 2).

The animal was euthanized and necropsied on day 10. Gross examination revealed marked swelling of systemic lymph nodes, including peripheral lymph nodes, and both abdominal and thoracic cavities. Discrete white masses of various sizes were also found in the kidneys, ribs, intracranial dura mater, compressed cerebrum, nasal septum, and frontal sinus. We observed a yellowish brown gelatinous material that accompanied synovial villous hypertrophy periarticularly in both carpal and stifle joints (Fig. 3).

Histopathological examination revealed neoplastic lymphoid cell with large round nuclei and scant amounts of cytoplasm infiltration in the enlarged lymph nodes, liver, uterus, heart, and pulmonary pleura. The synovial membrane was infiltrated with numerous neoplastic lymphocytes and showed papillary projections consistent with macroscopic findings (Fig. 4). Neoplastic cells were also found in the leptomenix and perivascular spaces of the cerebrum. Immunohistochemical examination of the enlarged lymph nodes revealed that tumor cells stained positive for CD3 and negative for BLA-36 antibodies, respectively.

From these findings, the present case was classified as calf type B-cell bovine lymphoma, as proposed by Yin *et al*. [17]. Although tumor cells are often observed in various organs in calf form bovine leukosis [11, 14], infiltration into joints and periarticular tissues is quite rare. Only one case of ataxia by tibiotarsal joint infiltration of tumor cells in calf form bovine leukosis has been reported [12]. The present case was a rare clinical case of multiple joint swelling related to calf form bovine leukosis, and although no neurological signs were observed, tumor masses in the cranial cavity compressed the cerebrum.

Activities of total LDH, LDH-2, and LDH-3 have been used as markers of enzootic bovine leukosis [6, 8]; however, the specificity is not high enough to confirm a diagnosis of lymphosarcoma. The present case showed slightly higher activities of total LDH, LDH-2, and LDH-3. Serum TK activity has also been used as a bovine leucosis biomarker [13], and the TK activity of the present case was higher than established values for healthy animals (5.4 IU/*l*) [13]. These combined results suggest that measurement of activities of both serum LDH isozymes and TK can be used as markers of calf form bovine leukosis as well as enzootic bovine leukosis.

IL2R is a heterotrimeric protein expressed on the surface of certain immune cells, such as lymphocytes and natural killer cells, and binds to interleukin 2 [10, 16]. TK is a cellular enzyme involved in a DNA synthesis salvage pathway, and its levels are directly correlated with tumor cell proliferation [4, 7]. Both *IL2R* and *TK1* genes are overexpressed in several human leukemia cases [2, 9]; however, there are no reports available for bovine leukosis. In the present case, these 2 genes showed increased expression compared to control cattle. More cases will need to be assessed to determine the validity of using these genes as markers of bovine leukosis.

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