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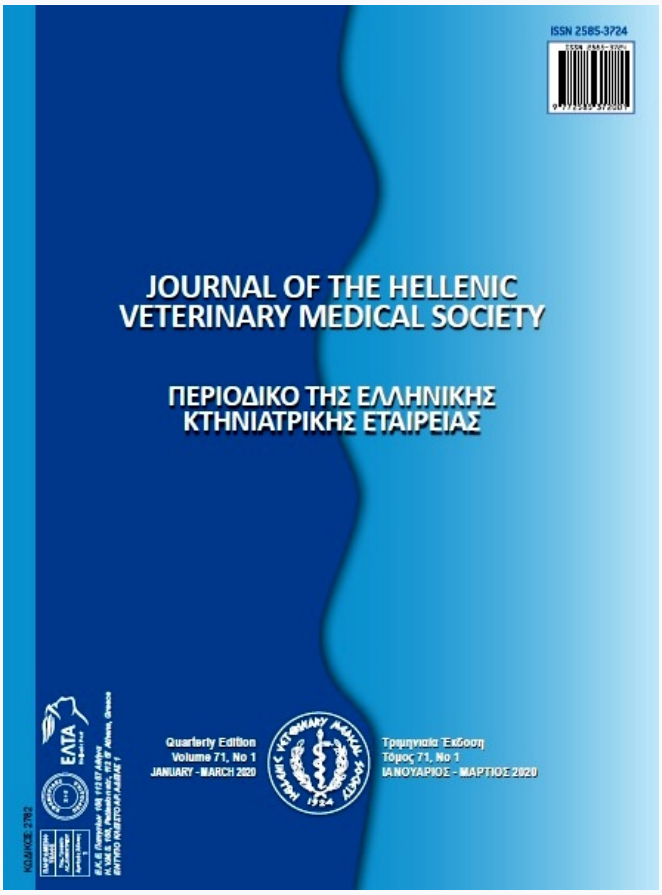

Enzootic bovine leukosis accompanied by splenomegaly in an 8-month-old calf

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Case report
Κλινικό περιστατικό

Enzootic bovine leukosis accompanied by splenomegaly in an 8-month-old calf

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ABSTRACT. In this report, an 8-month-old calf (crossbred, Holstein × Japanese Black) developed fever and accompanied abomasum displacement. Blood chemical test showed remarkably high values of white blood cell count and heteromorphic lymphocytes. In pathological appraisal, enlarged splenomegaly and swelling of the lymph nodes were observed. Histopathological examination revealed invasion of tumor cells derived from B1 cells into systemic lymph nodes, liver and spleen. The provirus loads of bovine leukemia virus (BLV) was 1,439 copies per 10 ng DNA by using real time PCR. In conclusion, this case was diagnosed as bovine leukemia caused by BLV infection with a huge splenomegaly.

Keywords: BLV virus, juvenile, splenomegaly.

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CASE HISTORY

Bovine leukemia is classified into two types: sporadic bovine leukosis (SBL) and enzootic bovine leukosis (EBL) (Kettmann, 1994). SBL is a generalized lymphadenopathy in calves, including a thymic form which is developed in 6-month to 2-year-old cattle and a cutaneous form occurred in 1- to 3-year-old cattle. EBL is a contagious disease caused by infection with bovine leukemia virus (BLV) which is belonging to the genus Deltaretrovirus, subfamily Orthoretroviridae, and family Retroviridae (Sagata et. al., 1985). Most BLV-infected cattle are asymptomatic infections (Aleukemia: AL) (Rodríguez et. al., 2011), but 20 to 30% of infected cattle show persistent lymphocytosis (PL) with polyclonal B cell hyperproliferation. Furthermore, 2 to 3% of them develop a B-cell leukemia, malignant lymphosarcoma formed in the lymph nodes, leading cause of death (Schwartz 1994). The incidence of EBL is observed more frequently in adult cattle over the age of 3 years than young calf. (Gutiérrez et. al., 2014). However, we report a suspicious case of EBL in 8-month-old calf which showed remarkable splenomegaly in this report. Our aim is description and thorough investigation of the clinical case.

An 8-month-old calf (castrated male, crossbreed, Holstein × Japanese Black) was examined because of decreased appetite and cough. The calf showed several symptoms such as abdominal pain, dehydration, fever (39.9 °C), and high respiration rate (60 breaths per min). In addition, it had been treated with enrofloxacin (Baytril®, Bayer, 0.025mL/kg, subcutaneously, SID) in order to control a suspected pulmonary inflammation. The serum hematological parameters provided a high leukocyte count (600,500 per 1 μL blood, reference interval (RI) : 4,900-12,000 per 1μL blood) and a percentage of heteromorphic lymphocytes was 90.5%. Moreover, lactate dehydrogenase (LD) and gamma-glutamyl transpeptidase (γ- GTP), the serum biochemical parameters, also showed high values (LD: 4,203 U/L, RI : 697-1,445 U/L, γ- GTP: 138 U/L, RI : 15-39 U/L).

After the calf was sedated with xylazine (Seractal®, Bayer, 0.3mg/kg, intramuscularly, SID) and euthanized after anesthesia with pentobarbital (Somnopentyl, Kyoritsu Pharmaceutical, 2mg/kg, intravenously) compassionately because of poor prognosis, the pathological autopsy was conducted, and we found a remarkable splenomegaly (93 × 36 cm, the weight of about 14 kg, Fig.1). Each of the lymph nodes (bilateral lateral cervical, lower ilium and mesentery) was enlarged and its cleavage surface showed grayish white.

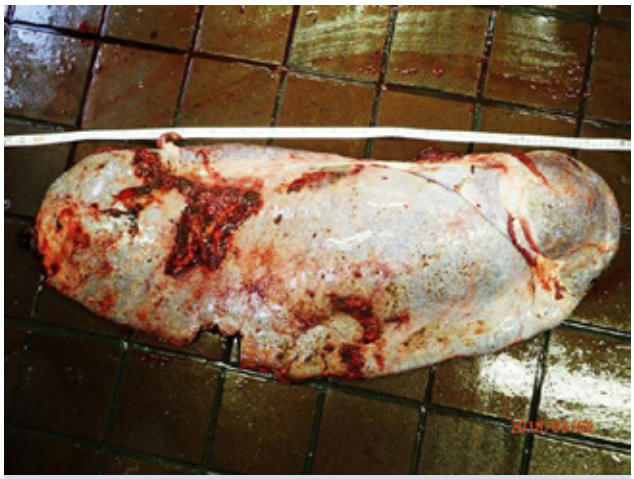

Figure.1: A huge splenomegaly (93 × 36 cm, the weight of about 14 kg). Necrosis and bleeding were observed.

The collected tissue samples were fixed with 10% neutral buffered formalin solution, embedded in paraffin, cut into thin sections and stained with hematoxylin and eosin (HE). As histological findings, tumor cells which showed mantle zone histological patterns and nodular or diffuse histological patterns were observed in each lymph node and spleen (Fig.2). Extracapsular invasion was rarely seen in lymph nodes. Additionally, the tumor cells were also observed in blood vessels of liver (Fig.3), and high magnification view of that area showed a monotonous proliferation of tumor cells (Fig.4). The tumor cells had round, oval or slightly irregular nuclei with inconspicuous nucleoli and cytoplasm which was moderately abundant.

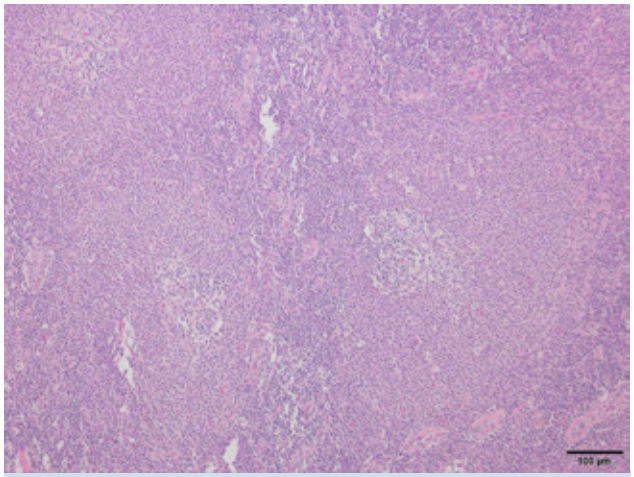

Figure.2: Histological appearance of iliac lymph node. Tumor cells which showed mantle zone histological patterns were observed. Hematoxylin and eosin (HE) staining.

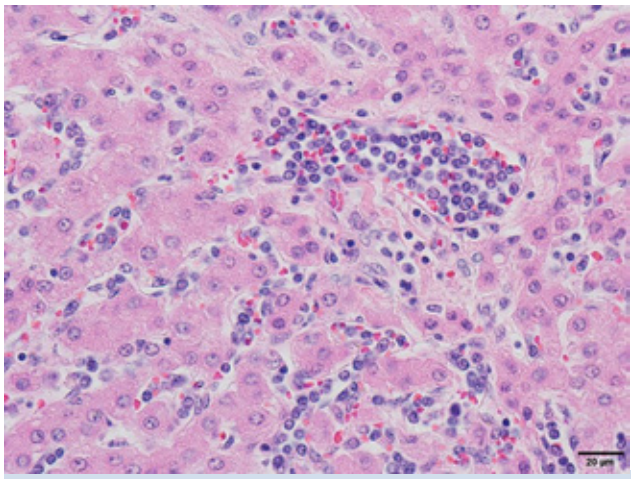

Figure.3: Histological appearance of liver tissue. Tumor cells were observed in blood vessels and sinusoid. HE staining.

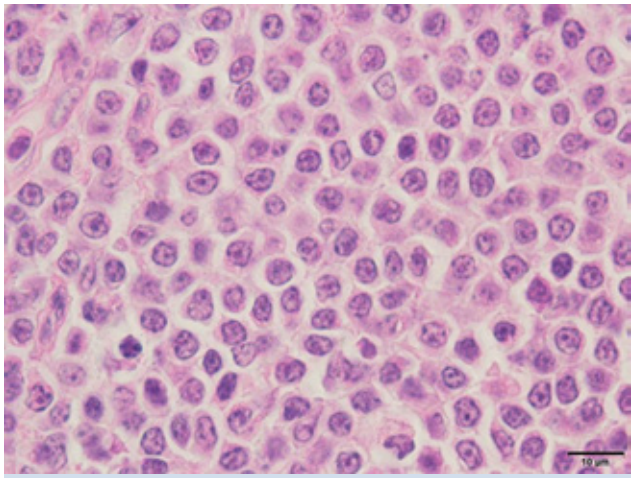

Figure.4: Histological appearance of iliac lymph node. A monotonous proliferation of tumor cells were observed. There were round, oval or slightly irregular nuclei with inconspicuous nucleoli and cytoplasm which was moderately abundant. HE staining.

Immunohistochemical examination was carried out using paraffin section of the tissue by polymer-based detection method according to the following procedures: Endogenous peroxidase activity was quenched by a solution of 0.3% hydrogen peroxide in methanol, after which antigen retrieval of the sections were performed by microwave heating. Antibodies targeting human CD20 (Thermo Fisher Scientific, Waltham, MA), human CD5 (Thermo Fisher Scientific), human CD3 (Agilent Technologies Japan, Tokyo, Japan) and TdT (Nichirei Biosciences, Tokyo, Japan) were used as primary antibodies. As results, the tumor cells expressed CD20 and CD5, but not CD3 and TdT (Fig.5).

To confirm the expression levels of cell surface markers in more detail, flow cytometry analysis was conducted using peripheral blood mononuclear cells prepared by density-gradient centrifugation. Cells were stained with following antibodies according to the method previously described (Nishimori et. al., 2017): bovine CD5 (Washington State University Monoclonal Antibody Center, Pullman WA; CAC-T105A), bovine IgM (Bio-Rad Laboratories, Hercules, CA; IL-A30), bovine WC4 (Bio-Rad Laboratories; CC55), bovine CD21 (Washington State University Monoclonal Antibody Center; GB25A), bovine CD3 (Washington State University Monoclonal Antibody Center; MM1A), bovine CD4 (Bio-Rad Laboratories; CC30) and bovine CD8 (Bio-Rad Laboratories; CC63). Alexa Fluor 647-conjugated goat anti-Mouse IgG (H+L) antibody (Thermo Fisher Scientific) was used as a secondary antibody, and for IgM staining, antibody labeling was conducted by Zenon Alexa Fluor 488 mouse IgG1 labeling kit (Thermo Fisher Scientific). The results clearly indicated the single cell population expressing CD5, IgM and CD21, but not expressing other markers (Fig.6).

To quantify BLV provirus loads in peripheral blood, genomic DNA was extracted by using an automatic nucleic acid extractor (magLEAD12gC, Precision System Science, Chiba, Japan) and BLV real time PCR was performed with Probe / Primer / Positive control for bovine leukemia virus detection (TaKaRa Bio, Otsu, Japan) (Soumura et. al., 2007). The result showed a quite high value of BLV provirus loads, 1,439 copies per 10 ng DNA, suggesting an interaction between tumor development and BLV infection.

The DNA extract was also used for B-cell clonality analysis, nested PCR targeting a variable region of immunoglobulin heavy chain, according to the method previously described (Nishimori et. al., 2017). As a result, we confirmed a single DNA band of the amplicon against the target region demonstrating monoclonal proliferation of B cells (Fig.7).

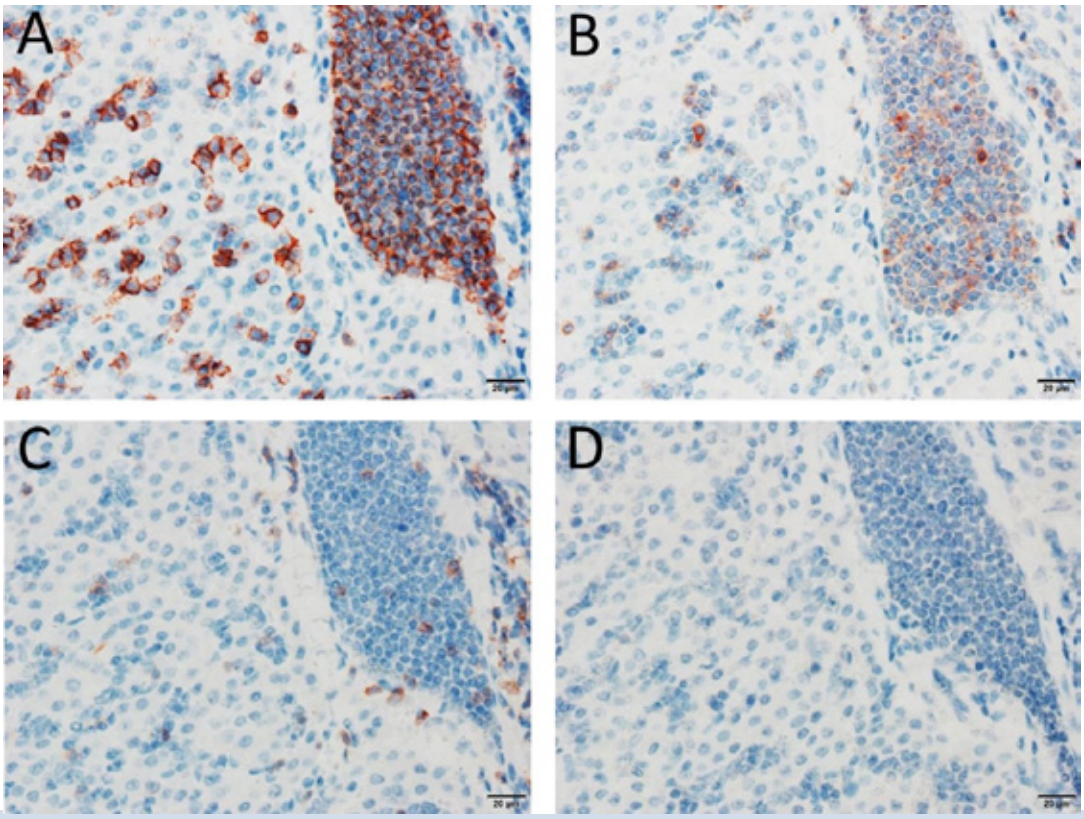

Figure.5: Immunohistochemical examination of tumor cells in liver tissue. Tumor cells were CD20 positive (A), CD5 positive (B), CD3 negative (C), TdT negative (D).

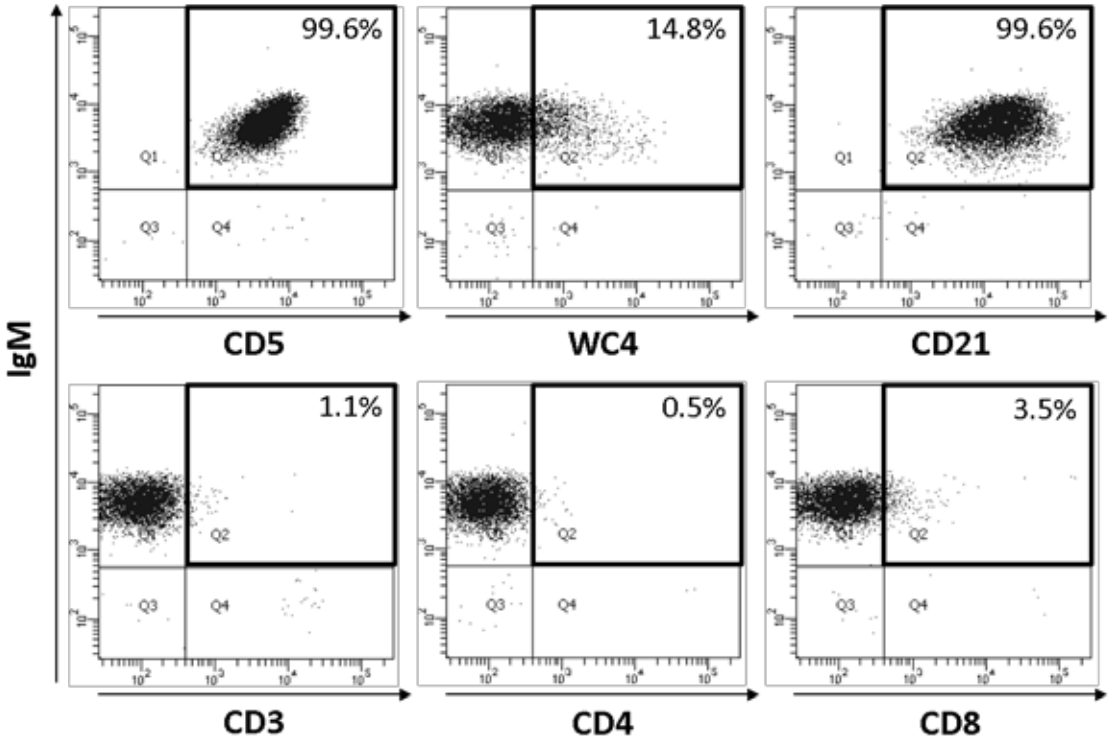

Figure.6: Flow cytometric analysis of peripheral blood mononuclear cells. Cells were first stained with anti-CD5 monoclonal antibody (mAb), anti-WC4 mAb, anti-CD21 mAb, anti-CD3 mAb, anti-CD4 mAb and anti-CD8 mAb, followed by Alexa Fluor 647-conjugated secondary antibody. Then cells were stained with Alexa Fluor 488-labeled anti-IgM mAb for double staining. The numbers in the figure indicate the percentages of double positive cells, and peripheral blood mononuclear cells showed CD5 positive, IgM positive and CD21 positive.

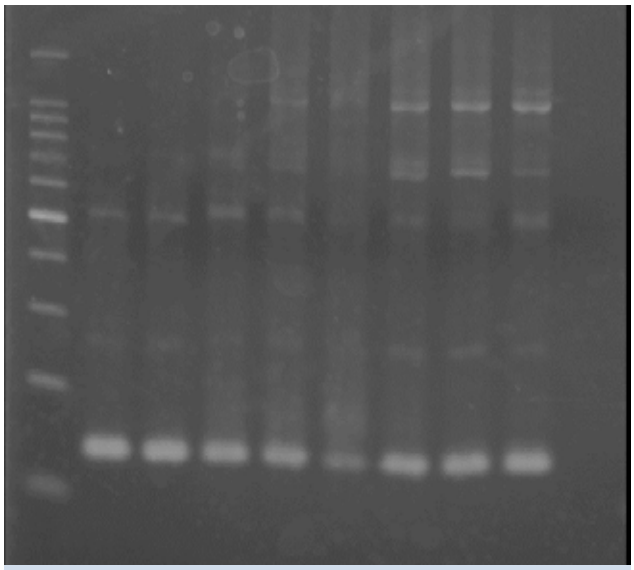

Figure.7: A single band patterns for the target region demonstrating monoclonal proliferation of B cells. Lane 1, white blood cells; lane 2, peripheral blood mononuclear cells; lane 3, a superficial cervical lymph node; lane 4, an iliac lymph node; lane 5, a mesenteric lymph node; lane 6, a mediastinal lymph node; lane 7, a pancreatico-duodenal lymph node; lane 8, spleen; N, negative control. The arrow indicates PCR amplification of the target region.

DISCUSSION

In previous studies, it was reported that the infection rates of BLV in Japan had increased from around 5% in the 1980s to 35.2% on a nationwide survey conducted in 2009-2011 (Murakami et. al., 2013). Thus, farmers suffer economic losses due to development of bovine lymphoma causing not only death of cattle, but also large costs for treatment and diagnosis of the infected animals (Da et. al., 1993). Early detection of cattle infected with BLV is effective for preventing BLV-free cattle from the infection and reducing economic losses. Moreover, an epidemiological study also suggests an importance of controlling BLV infection; it demonstrates that the incidence of other infectious diseases increases in BLV infected cattle compared with uninfected cattle (Emanuelson et. al., 1992).

Currently, little is known about the occurrence of EBL in young cattle under 1 year of age, and only a few reports investigate about that (Oguma et. al., 2017). Moreover, complication of splenomegaly is infrequent in common EBL cases. So, we consider that our report will contribute to accumulation of knowledge on bovine leukemia in young cattle and will be helpful for the diagnosis in the field. In this report, it was not able to investigate whether mother-to-child transmission of BLV had occurred or not because the dam of the case animal had already slaughtered. However, according to previous reports, almost all calves that are infected with BLV immediately after birth are likely to develop into PL (Agresti et. al., 1993), and probability of BLV transmission in utero is around 4% to 18% (Ferrer et. al., 1975). Additionally, in the case of calves whose mother showed high provirus loads, more than 40% of the infants have been confirmed BLV positive whereas BLV positive rates were only 9.4% in the calves born from mothers with low provirus loads (Mekata et. al., 2015). In this case, we consider that BLV infection could be induced by vertical transmission via colostrum or placenta rather than by horizontal transmission via insects because the calf showed quite high value of white blood cell count and BLV provirus loads. Moreover, we concluded that the tumor cells in this case were derived from B1 cells, which is consistent with a previous report investigating BLV-associated lymphomas in cattle (Vernau et. al., 1997). Additionally, a single DNA band of the amplicon against the target region demonstrated monoclonal proliferation of B cells (Fig.7).

Because of the entire results we presented above, this case was diagnosed as EBL, which is clonal expansion of tumor cells derived from B1-cell lineage due to BLV infection, in spite of the young age of the animal. This finding is different from a widely accepted belief that EBL occurs in mainly adult cattle.

It is known that lymphoid tumor in adult lymphoma usually has a predilection for heart, abomasum, uterus, kidney, and spinal cord (Burton et. al., 2010). However, tumor invasion into those organs was not confirmed in this case, but instead, invasion into liver, spleen and lymph nodes was observed. In particular, the splenomegaly was one of the most important finding because it is infrequent in common EBL cases. There are some reports about the incidence of splenomegaly in cattle induced by Anaplasma marginale infection (Jaswal et. al., 2015), hemophagocytic histiocytic sarcoma (Matsuda et. al., 2010), and Trypanosoma vivax infection (Fatihu et. al., 2008). In this case, the parasites listed above were not observed in histological examination. Bovine leukemia is grouped into malignant lymphoma, and in our case, tumor cells would grow in the spleen, which caused enlarged splenomegaly.

There is still limited information about the onset of EBL in juvenile cattle. Further study on BLV infection and its dynamics during young stage would be necessary to elucidate its detailed mechanism.

CONFLICT OF INTEREST

None declared.

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