

Short Communication

JUVENILE LYMPHOSARCOMA IN CALF: A CYTOHISTOLOGICAL ANALYSIS

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ABSTRACT: Bovine lymphosarcoma is one of the most prevalent neoplastic diseases in cattle, which affects both dairy and beef animals. Serological evidence and molecular characterization of Enzootic bovine leukemia are reported from India, but reporting of juvenile lymphosarcoma in the calf is scarce. A four-month-old female HF Cross calf with depression, progressive weight loss, weakness, and enlarged peripheral lymph nodes were brought for treatment to the Veterinary Clinical Complex (VCC), SKUAST-J, Jammu, R.S. Pura, India. Peripheral blood showed leukocytosis, lymphocytosis, and normocytic, normochromic anemia. Blood smear and cytopathological examinations revealed the presence of atypically large lymphocytes with obvious clear cytoplasmic vacuoles and cleaved, reniform, amoeboid nuclei with unclear nucleoli. Histopathological examination of the affected lymph nodes showed the presence of a tumor that was characterized by proliferating round, distinct pleomorphic cells having minimal cytoplasm, prominent nuclei, and oval and round basophilic shapes. On the basis of cytological and histo-pathological basis, the present case was described as sporadic juvenile lymphosarcoma.

Key words: Juvenile bovine lymphosarcoma, Peripheral blood, FNAC, Histopathology.

One of the most prevalent neoplastic diseases in cattle, which affect both dairy and beef animals, is bovine lymphosarcoma (BLS). On the basis of epidemiological and pathological data, two forms of the disease can be categorized based on the organ systems involved, age at onset, frequency of occurrence, and causative agent (Nasir 2005). Cattle are affected by two kinds of leukemia/leukosis: Enzootic Bovine Leukaemia (EBL) and Sporadic Bovine Leukaemia (SBL). The bovine leukemia virus (BLV) is responsible for the “adult form” of Enzootic Bovine Leukosis. Younger animals (under 3 years of age) are more susceptible to sporadic bovine leukosis, which develops independently of BLV infection. The calf or juvenile, thymic or adolescent, and skin or coetaneous are the types of SBL that have all been described in cattle (Johnson 1998). While SBL may be the result of either B-lymphocytes or T-lymphocytes undergoing a malignant mutation, enzootic bovine leukosis is a tumor of B-lymphocytes (Peleteiro *et al.* 2000). In calves younger than six months old and occasionally in some fetuses, the calf or juvenile variant of SBL manifests widespread

lymphadenopathy. This type of SBL is rare, has an unknown frequency rate, and is more likely to develop in dairy breeds (Miller 1993). Serological evidence (Bansal *et al.* 1980, Bansal and Singh 1981) and molecular characterization (Gautam *et al.* 2018) of BLV strains in cattle in India have been reported earlier, however, to the best of our knowledge, no information existed on the occurrence of juvenile sporadic bovine leukosis (SBL) in the calf. Histopathology is considered the gold standard for the detection and staging of many cancers, treatment-related changes, and many other situations at the tissue level (Jisna *et al.* 2021, Pandey *et al.* 2021, Jain *et al.* 2022). The present report clinically and pathologically describes a juvenile sporadic case of lymphosarcoma in a bovine calf.

The study

A four-month-old female Holstein Friesian Cross calf with depression, progressive weight loss, weakness, and enlarged peripheral lymph nodes, was brought for treatment to the Veterinary Clinical Complex (VCC),

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SKUAST-J, Jammu, R.S. Pura, India. Bilaterally enlarged retropharyngeal, submandibular, pre-scapular, and pre-femoral lymph nodes were observed during physical examination (Fig. 1). After a week of treatment with non-steroidal anti-inflammatory medicines and broad-range antibiotics, the animal showed no signs of recovery. The results of the rest of the physical examination were all within normal ranges. On the basis of clinical signs and gross enlargement of peripheral lymph nodes, a tentative diagnosis of sporadic juvenile lymphosarcoma was made. This was confirmed by fine needle aspiration cytology (FNAC) of an enlarged retropharyngeal lymph node. A 20 mL syringe and a 23-gauge needle were used to do routine FNAB. Cytological smear was prepared and stained with Wright-Giemsa as per the standard method described (Benjamin 2001). Under aseptic conditions, a tissue biopsy from the retropharyngeal lymph node for histopathology was done. For histologic analysis, tissue samples were routinely treated and stained with hematoxylin and eosin after being preserved in 10% neutral buffered formalin. The samples were then dehydrated and mounted on slides using DPX mountant after being counterstained with Harris Hematoxylin for 30 seconds at room temperature and observed under an optical microscope for recording histopathological changes (Bancroft and Gamble 2002). Peripheral blood was also collected for the estimation of blood parameters as per the standard method (Benjamin 2001).

Results and discussion

Peripheral blood showed leukocytosis, lymphocytosis, and normocytic normochromic anemia (decreased PCV, RBC, hemoglobin) and the presence of atypically large lymphocytes with obvious clear cytoplasmic vacuoles and

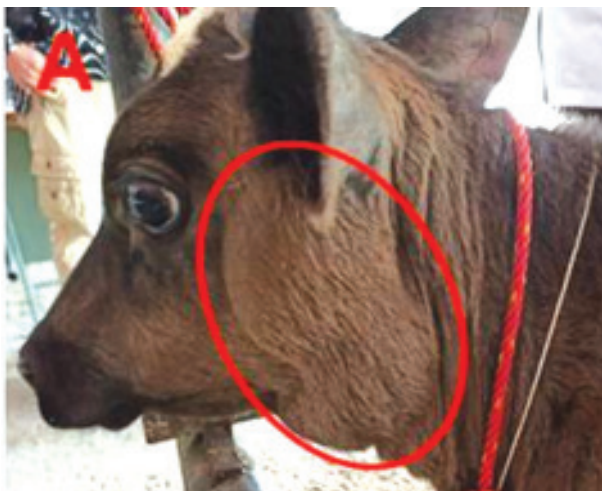


Fig. 1. Bilateral enlargement of retropharyngeal lymph node.

cleaved, reniform, amoeboid nuclei with unclear nucleoli. The fine needle aspirates' cytological examination revealed strong cellularity and high cell fragility. A monomorphic population of big lymphoblasts was seen in the smear, with high nuclear to cytoplasmic ratios, more basophilia, single to multiple discrete nucleoli, and highly stippled chromatin patterns. Large lymphocyte nuclei ranged in form from round to oval to kidney-shaped or had a deep depression. Most of the lymphoblasts had many eosinophilic granules in their cytoplasm, and a small number had bi- or tri-nucleus (Fig. 2).

Histopathological examination of the affected lymph node showed tumors characterized by proliferating round, distinct pleomorphic cells with minimal cytoplasm, prominent nuclei, and oval and round basophilic shapes. These cells were also occasionally found to have loose chromatin and prominent nucleoli. These malignant lymphocytes had a diffuse proliferation that was dispersed throughout a large solid pattern; in some spots, a thin connective tissue strand separated the neoplastic cells. Neoplastic cells either totally or partially changed the lymph nodes' natural architecture. The cortical and medullary architecture was almost completely destroyed. The tumor masses also displayed neoplastic cell vascular invasion and the occasional presence of mitotic figures (Fig. 3A and Fig. 3B).

The term "lymphoma" is used to denote solid lymphocyte neoplasms. Lymphoid neoplasia, also known as lymphosarcoma or malignant lymphoma, has been described in cattle (Johnson 1998). SBL affects animals

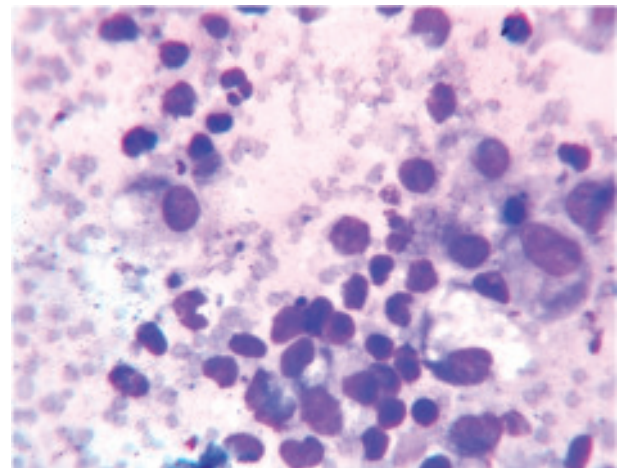


Fig. 2. Fine needle aspirates' cytological examination of blood. [A monomorphic population of large lymphocyte of which nuclei were ranged in form from round to oval to kidney-shaped with high nuclear to cytoplasmic ratios, more basophilia, single to multiple discrete nucleoli, and a highly stippled chromatin patterns. Wright-Giemsa X1000].

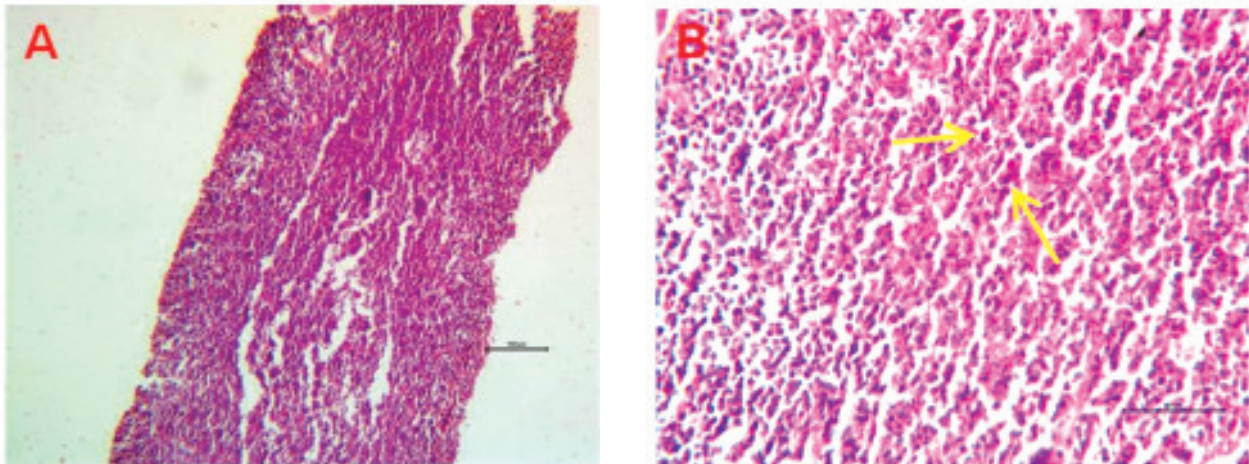


Fig. 3. Histopathological examination of affected retropharyngeal lymph node.

[(A) Cortical and medullary architecture was almost completely destroyed by neoplastic lymphocytes. H&E X100. (B) These malignant lymphocytes were round, distinct pleomorphic cells having minimal cytoplasm, prominent nuclei, and oval and round basophilic shapes that were dispersed throughout as a large solid pattern; in some spots, a thin connective tissue strand separated the neoplastic cells with the occasional presence of mitotic figures (Yellow arrows). H&E X 400].

younger than three years old and is generally believed not to be related to the bovine leukemia virus (BLV), unlike EBL that has an incubation period of about four to five years and is caused by the BLV (Radostits *et al.* 2007). Rarely diagnosed, sporadic bovine leukosis (SBL) is thought to infect 1 animal per 100,000 cattle (Oliver-Espinosa *et al.* 1994). It is noncontagious, nontransmissible, and typically only found in young cattle, usually those under a year old as a single case in a herd. Incomplete Bovine sarcoma virus fragments might induce sporadic bovine leukosis, however, no virus has been isolated or shown by electron microscopy, and antibodies to BLV have not been found (Chander *et al.* 1977).

According to Harbo *et al.* (2004), Washington State University received a two-month-old Angus heifer suspected of sporadic juvenile lymphoma, and the clinical history and histological results in the present case study closely matched descriptions of this reported juvenile SBL. Compared to EBL, pathologically juvenile SBL has less common involvement of the heart, abomasum, and uterus. It also features symmetrical, multicentric lymph node swelling (Bundza *et al.* 1980). The increase in total leukocyte and lymphocyte counts observed in the present study was consistent with those reported in the literature (Theilen and Dungworth 1965, Hugoson 1967). Similar clinical signs, cytological and histopathological changes in sporadic bovine leukosis in a 5-to-6-month-old Holstein heifer were reported by Oliver-Espinosa *et al.* (1994). De Oliveira *et al.* (2016) reported for the first time, lymphoma in four buffaloes in the Amazon region of Brazil in 2016, along with gradual weight loss, swollen peripheral

lymph nodes, and lumps in the subcutaneous tissues. Histopathologically, proliferated neoplastic lymphocyte was observed in nodules. Hassan *et al.* (2018) presented a 7-year-old crossbred Holstein-Friesian cattle with an unusual spontaneous clinical instance of persistent diarrhea caused by lymphosarcoma. Aspiration of peritoneal fluid, lymph node enlargement, and a lack of fecal or serological evidence of any parasite or bacterial infection all contributed to the clinical diagnosis of lymphosarcoma. Leukocytosis and a sharp drop in hemoglobin level, which indicated a severe degree of anemia, were discovered during a hematological examination. Despite recent efforts to better understand the condition, sporadic bovine leukosis continues to be a clinical and pathologic mystery. It is far less prevalent than enzootic bovine leukosis, and among the three types that are known, the cutaneous form has been reported the most frequently, while the juvenile and thymic form has been reported the least frequently (Jones *et al.* 1997).

Despite all of the clinical and pathological alterations, a BLS diagnosis cannot be made with certainty without a histopathologic analysis of the affected tissues. To exclude granulomatous disease or other forms of tumor that could cause similar gross tissue alterations, histopathologic confirmation is required (La Follette and Davis 1994).

Conclusion

In the present study, a four-month-old female HF Cross calf with depression, progressive weight loss, weakness, and enlarged all peripheral lymph nodes were brought for treatment to the Veterinary Clinical Complex (VCC),

SKUAST-J, Jammu, R.S. Pura, India, and peripheral blood showed leukocytosis, lymphocytosis, and normocytic normochromic anemia. Blood smear, cytological and histopathological examinations revealed the presence of atypically large lymphocytes with obvious clear cytoplasmic vacuoles and cleaved, reniform, amoeboid nuclei with unclear nucleoli. On the basis of cytological and histopathological basis, the present case was described as sporadic juvenile lymphosarcoma.

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