



Case Report

Spleenic Lymphoma and Hemoperitoneum in Jersey Bull: Clinical and Pathological Aspects

Paulo Henrique Sampaio¹, Otávio Luiz Fidelis Junior¹, Pamela Rodrigues Reina Moreira¹,
Lúcia Helena Rodrigues¹, Fabiano Antonio Cadioli³, Rosemeri de Oliveira Vasconcelos^{2*},
Luiz Carlos Marques²

¹Graduate student of FCAV – Unesp/Jaboticabal

²Professor of FCAV – Unesp/Jaboticabal

³Professor of FMVA – Unesp/Araçatuba

* Corresponding Author: UNESP/Jaboticabal, Department of Veterinary Pathology, 14884-900, Jaboticabal/SP, Brazil. E-mail: rosevasc@fcav.unesp.br

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Abstract

Lymphoma is the most prevalent neoplasia in dairy cattle. The etiology can be viral in animals affected by bovine leukemia virus (BLV) or be classified as primary. Lymphoma can affect several organs and according to the system involved, the clinical signs could manifest themselves in different ways. These tumors can be classified through macroscopic characteristics, histology and immunostaining. This classification can be used to predict prognosis and response to therapy. The aim of this case report was to immunostain and classify the tumor, for which anti-CD4, anti-CD8, anti-CD79 and anti-CD3 markers were used in addition to histopathological findings, in order to classify the tumor. The tumor was positive only for anti-CD3 marker, indicating that it is a tumor of young cells and, in association with histopathology and hematological data, it can be concluded that spleen neoplasia is lymphocytic lymphoma originated from a lymphocytic leukemia.

Key words: immunophenotyping, bovine, CD3, lymphocytosis.

Introduction

Lymphoma is the most prevalent neoplasia in dairy cattle. It has been observed a greater incidence in older animals, and the most affected organs are the lymph nodes, heart, abomasum, duodenum, kidney, uterus, epidural area of the lumbar vertebra, retrobulbar area and spleen. Thus, symptoms vary according to the compromised organ (3).

Spleen affected by lymphoma may result in rupture, leading to death due to hemorrhage (11; 7). In cattle, the bovine leukemia virus (BLV), trauma and other types of spleen neoplasia, can produce similar clinical signs (9). The lymphoid leukemia not associated with BLV is rare in bovines (14), in this species the lesions occur in

the bone marrow, and when metastases occur they are more common in the spleen, liver and lymph nodes, usually in advanced stages of the disease (15). Immunophenotyping of lymphomas in adult cattle has the predominance of B lymphocytes, whereas in sporadic cases there may be B or T lymphocytes (3).

In literature, there are few data regarding immunophenotyping of lymphoma in bovine spleen associated to lymphoid leukemia. The objective of the present study is to characterize neoplasia through immunohistochemistry, histopathology and hematology, and subsequent correlation with prognosis. It is possible that this location is correlated with poor prognosis of the disease, justifying the present report.

Case Report

An eight year-old Jersey bull was found in lateral decubitus in the bay, having difficulty to be stand up. On the previous day, the animal was subjected to semen collection. The procedure was successful and no abnormalities were detected.

The animal was subjected to physical examination. It was in sternal recumbence showing pronounced tachypnea, distended abdomen, the skin and extremities were cold. The animal was looking for the right flank (abdominal discomfort) and it also presented slightly pale mucosal, high heart rate (120/min), intense breathing (80 movements/min) and a temperature of 38°C. Only secondary movements of rumen were preserved. Once stimulated, the animal got up with difficulty and presented noticeable muscle weakness, characterized by staggers and tremors. After given guidance, the animal showed reluctance to move, stayed a few seconds on station and laid down again. Once the animal was treated with analgesics and fluid, it presented an apparent improvement, especially with regards to signs of abdominal distress. The patient died approximately four hours after being found recumbent.

A complete blood count (CBC) was performed four hours before death (Table 1) and a significant leukocytosis count (430,000/mm³) was observed as well as, eosinophilia, monocytosis, a high number of immature cells (blasts) and leukemia. Blood smear can be observed in Figure 1.

Table 1. A CBC of Jersey bull with splenic lymphoma.

Parameter	Relative Value (%)	Absolute Value/mm ³	Reference Values (9)
Leukocytes	-	430,000	4,000-12,000
Blasts	25	107,500	0
Neutrophils	1	4,300	600-4,000
Eosinophils	1	4,300	0-2,400
Lymphocytes	71	305,300	2,500-7,500
Monocytes	2	8,600	25-840
Platelets (/μl)	-	325,000	100,000-800,000
RBC (x10 ⁶ /μl)	-	3.54	5.0-10.0
Hemoglobin (g/dL)	-	8.5	8-15
PCV (%)	25	-	24-46
MVC (fL)	70.6	-	40-60
MCHC (g/dL)	-	34	30-36

In the abdominal cavity, the presence of bloody content rich in large blood clots (hemoperitoneum) was observed and it was distributed throughout all organs (Figure 2). The spleen was greatly enlarged (Figure 2), measuring 110 x 30 cm (center) and weighing 8.5 kg, it

was also friable, presenting an irregular surface, and in dorsoventral position, occupying the entire length of the diaphragm. In the mid-third of the organ, the absence of capsule was noted in an extension of approximately 20 x 20 cm, and the adjacent tissue was friable and presented a black color. After being sliced, a white protruding pulp with an aspect of "sago" was observed. Only the apex of the organ (about 20 cm) presented an evident unchanged aspect.

In the histological sections of the spleen, stained with hematoxylin-eosin (HE), it was observed a proliferation of high cell density, arranged in mantle cell diffuse, not encapsulated, and not grouped indistinct boundaries between delicate fibrous stroma. The cytoplasm of the cells were scarce, eosinophilic, with homogeneous and round nucleus, paracentral with dense chromatin, moderate anisokaryosis and an irregular nuclei form, without visualization of mitotic figures in 10 high-power fields, being the diagnosis of lymphocytic lymphoma (Figure 3A).

Immunohistochemistry was performed with monoclonal antibodies CD4 and CD8 (T lymphocyte markers, Novocastra, cod. NCL-L-CD4-176 and cod. NCL-CD8-295, respectively), CD79 (B lymphocyte marker, DAKO cod. M7051) and polyclonal CD3, a marker of T lymphocytes () using Streptavidin biotin peroxidases complex (LSAB kit, DAKO, cat. K0690). Dilutions of antibodies were 1:50. Antigen retrieval was performed by heat (Pascal pressure chamber, DAKO), with a sodium citrate 10 mM solution (pH 6.0). Blocking endogenous peroxidases was used with methanol solution and hydrogen peroxide (30 v/v, Merck) at 8%, for 20 minutes. Blocking of unspecific proteins was performed with a commercial product (Protein Block Serum Free, DAKO, cat. X0909). The chromogen was diaminobenzine (DAB, DAKO, cat. K3468). The CD3 had positive staining in the membrane (Figure 3B) and other antibodies were negative. Positive controls were provided by bovine lymph node sections and only tissue sections incubated with antibody diluents were used for negative control.

Discussion

The Jersey bull has survived without apparent clinical manifestations until the hypovolemic shock, due to acute hemorrhage triggered by disruption of the splenic capsule. Therefore, certainly the cause of death can be attributed to peripheral circulatory failure. Hemoperitoneum due to rupture of granulosa and splenic rupture assigned to atypical lymphoma in cows, with similar clinical signs to those observed in the Jersey bull, were described by Masseur et al. (6) and Movassaghi & Taghipour Bazargani (7).

The splenic lymphoma studied cannot be attributed to virus infection of Enzootic Bovine Leukosis (EBL), since the animal belonged to a Central Cattle Breeding Farm. Serology for enzootic leukosis was held periodically

(every six months), it was performed using VMRD's Bovine Leukemia Virus Antibody Test Kit (VMRD, Pullman, WA, USA), with a sensitivity of 98% and specificity of 100%, and the results were always negative. Moreover, persistent lymphocytosis may affect 30% of animals with enzootic leukosis and this lymphocytosis may be three or more deviations above the average and persist for more than three months, being B lymphocytes predominant. However, the number of lymphocytes in peripheral blood measured from the Jersey bull on the day of death was 305,300 per mm^3 , a significantly high value compared to those expected in cases of enzootic leukosis, which are usually around 15,000 per mm^3 (9).

Lymphocytosis has two common causes, one due to the animal's stress and the other as a result of lymphocytic leukemia. In the first case, the increase is subtle, slightly above the upper limits, and in the second case, the increase is exaggerated (12).

Histological examination of spleen enabled the identification of blasts and young leukocytes precursors markedly increased, although usually the absence of these cells into the bloodstream is normal. Thus, findings suggest that this is a case of lymphocytic leukemia. The hereditary deficiency of lymphocyte maturation can affect cattle, this defect being autosomal and recessive, affecting young animals (around fourth months old) (9), unlike this case, it was an adult bovine.

Splenic mass was measured, weighing 8.5 kg, far above what is considered normal for bovine. Spleen of zebu and crossbred Holstein of 340 kg was around 0.955 and 1.226 kg, respectively (1). Crossbred dairy cattle and Indubrasil weighing 480 kg presented spleen with 1.486 and 1.404 kg, respectively (2). Only the spleen was affected in the Jersey bull in this case.

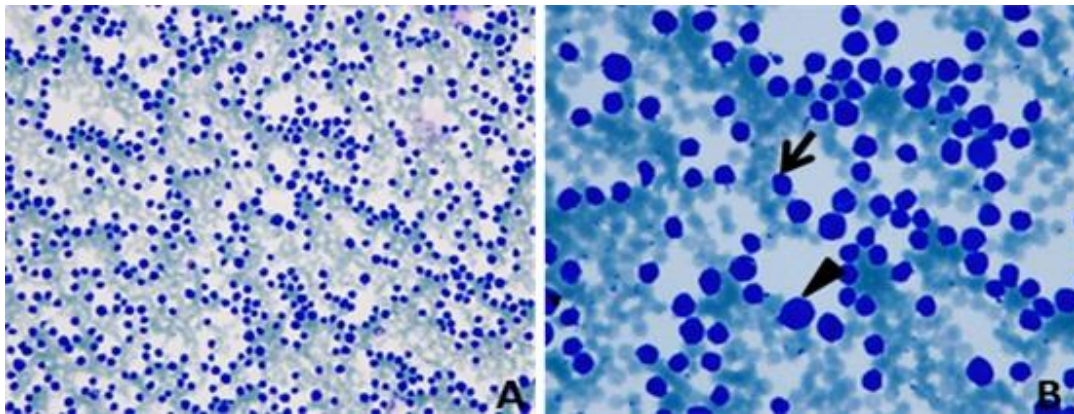


Figure 1. Jersey bull blood smear with spleen lymphoma. (A) Note high cellularity with predominance of lymphocytes (X400). (B) Note high cellularity, lymphocyte (arrow) and blasts (arrowhead) (X1000), diff quick stain.

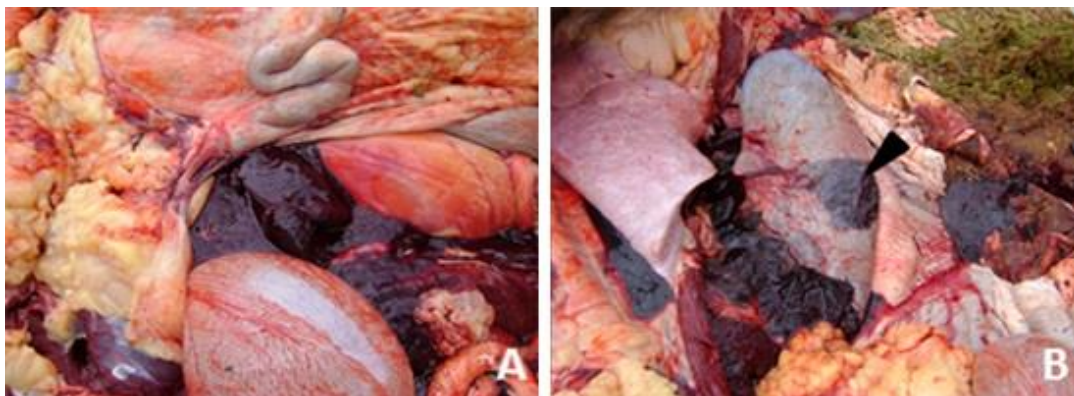


Figure 2. (A) Abdominal cavity of Jersey bull with spleen lymphoma. Note the presence of large blood clots (hemoperitoneum) distributed among the viscera. (B) Spleen of Jersey bull with lymphoma. Note large blackened areas with discontinuity of the capsule (arrowhead).

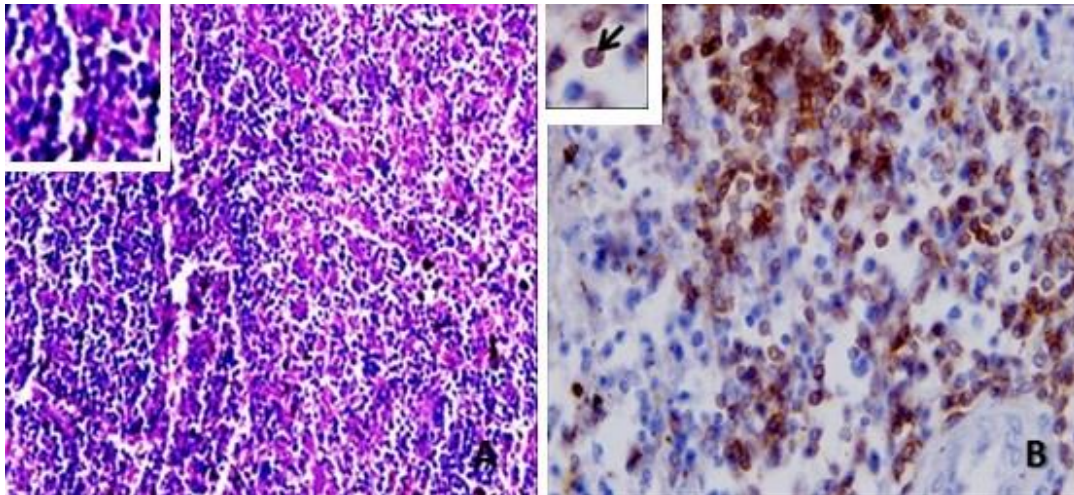


Figure 3. Photomicrograph of tumor mass of bovine with lymphocytic lymphoma. (A) Neoplastic proliferation of lymphoid cells that were invading the spleen, HE (X400). (B) Positive immunostaining of cytoplasmic membrane (arrow) with anti-CD3, Streptavidin Peroxidase Complex, hematoxylin counterstain (X1000).

Immunophenotyping was positive only for anti-CD3 polyclonal antibody, indicating that this was a tumor of immature cells, pro-T lymphocytes (5). In one case of thymic lymphoma, in a Nellore cow, only CD8 T lymphocytes were positively immunostained (10). Yamazaki et al. (13) held immunostaining of bovine lymphoma using human anti-CD3 polyclonal antibody (Dako), human anti-CD79 and anti-MHCII, only anti-CD3 being positive, similar to the present case. Kagawa et al. (4) e Murayama et al. (8) immuno marker lymphocytes B and T in different cases of lymphoma using anti-CD79a (Dako and WC1-N3 - Veterinary Medical Research and Development, Pullman, WA, USA), anti-CD3, TdT (Dako, Glostrup, Denmark) , anti-CD5 (Lab Vision, Fremont, CA, USA) antibodies, whereas immunostaining was varied. However, data on immunophenotyping of lymphomas in cattle are scarce in literature.

Conclusion

A lymphocytic lymphoma in spleen was the cause of splenic rupture followed by hemorrhage and hypovolemic shock. Histological analysis was consistent with lymphocytic lymphoma. Positive immunostaining only for anti-CD3 antibody certifies that this was a tumor of immature cells and, in association with the hematological findings the diagnostic of leukemia with lymphomatous lesions in the spleen, a rare disease in cattle.

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