



Case Report

Immunophenotyping of Thymic Lymphoma in a Nelore Cow

Paulo H. Sampaio¹, Otávio L. Fidelis Junior¹, Daniela J. de Queiroz¹, Luciana M. C. Soares¹, Pamela R. R. Moreira¹, Rosemeri O. Vasconcelos², Luiz C. Marques², Antonio C. Alessi²

¹Graduate student of FCAV – Unesp/Jaboticabal - Programa de Medicina Veterinária; ²Professor of FCAV – Unesp/Jaboticabal

* **Corresponding Author:** Rosemeri O. Vasconcelos, Department of Veterinary Pathology, UNESP/Jaboticabal. Via Prof. Paulo Donato Castellani, s/nº – Jaboticabal - SP, Brazil. 14884-900. e-mail: rosevasc@fcav.unesp.br

Submitted June 13th 2012, Accepted July 28th 2012

Abstract

Thymic lymphoma is a malignant lymphoid neoplasm that affects several species, including cattle. This type of neoplasia can lead to death due to malignant cell infiltration in different organs. The classification of this neoplasm may predict prognosis and response to treatment. Immunophenotyping is one of the ways to perform this classification. There are reports about the performance of immunohistochemistry (IHC) to classify thymic lymphoma only in taurine cattle, therefore the aim of this report is to describe the immunophenotype of a thymic lymphoma in a Nelore cow. Immunostaining was performed with monoclonal antibodies (CD79, CD4 and CD8). The tumor cells showed positive staining only for CD8 T lymphocytes, coinciding with the disease progression, since the T lymphoma type is more aggressive.

Key Words: Immunohistochemistry, thymic lymphoma, CD8 T cell, Nelore breed.

Introduction

Lymphoma can affect cattle and other species including humans. When this tumor has a high mitotic rate can quickly lead animals to death (5). The occurrence of lymphoma in adult cattle is more frequent, but also occurs in young bovines. Gender, race and seasonality have no influence on disease occurrence. All organs are potential targets for the tumor, but lymph nodes and heart are the most commonly affected (13). When there is direct or indirect involvement of the spinal cord neurological signs are evident (4).

One specific multicentric form occurs in calves up to six months, but in cattle up to 30 months the thymic form is predominant. In adults, the multicentric form is often enzootic, associated with the presence of the antibody and/or the Bovine Leukosis Virus (BLV). The incidence varies from two to 18 cases per 100,000 bovines (8). According to VALLI and PARRY (14) and Radostits et al. (12) thymic lymphosarcoma is a common finding in

1-2 year-old yearlings, being more frequent in beef cattle than in dairy cattle.

Despite many indications of a viral etiology, there are reports of this tumor in serologically negative animals for BLV (3, 7). Seronegative calves, sons of the same bull, developed thymic lymphoma probably with a genetic origin (11). In sporadic bovine leucosis, the virus cannot be detected and in this situation there is no evidence of an infectious cause (12). Even with reports about the lack of correlation between the BLV and thymic lymphoma, new studies demonstrated that the virus can be detected by PCR (2).

The type of tumor can predict response to the treatment and prognosis, and this is already a reality for humans and dogs. The classification schemes are based on the type of tumor cell. In cattle, the distribution of lymphoid tumor differs between young and adults, and in this species the age and the distribution of lesions imply in the etiology (5).

Cytological classification of lymphomas can be described as: well differentiated, with intermediate

differentiation, poor differentiation or large cell lymphoma; anatomical classification includes multicentric, thymic, alimentary, cutaneous and unique types; regarding immunohistologic phenotyping, they can be of type B, type T and null B/null T (8).

Sporadic juvenile lymphoma can occur in young bovines, below six months of age; there is a predominance of the type T cells over type B cells in this kind of tumor (10). In animals and humans, T cell tumors are more aggressive than those of B cell origin, showing lower response to therapy (5). Immunophenotyping studies have been conducted in cattle (15, 6, 9), however there is shortage of studies of this type of tumor in Nelore cattle.

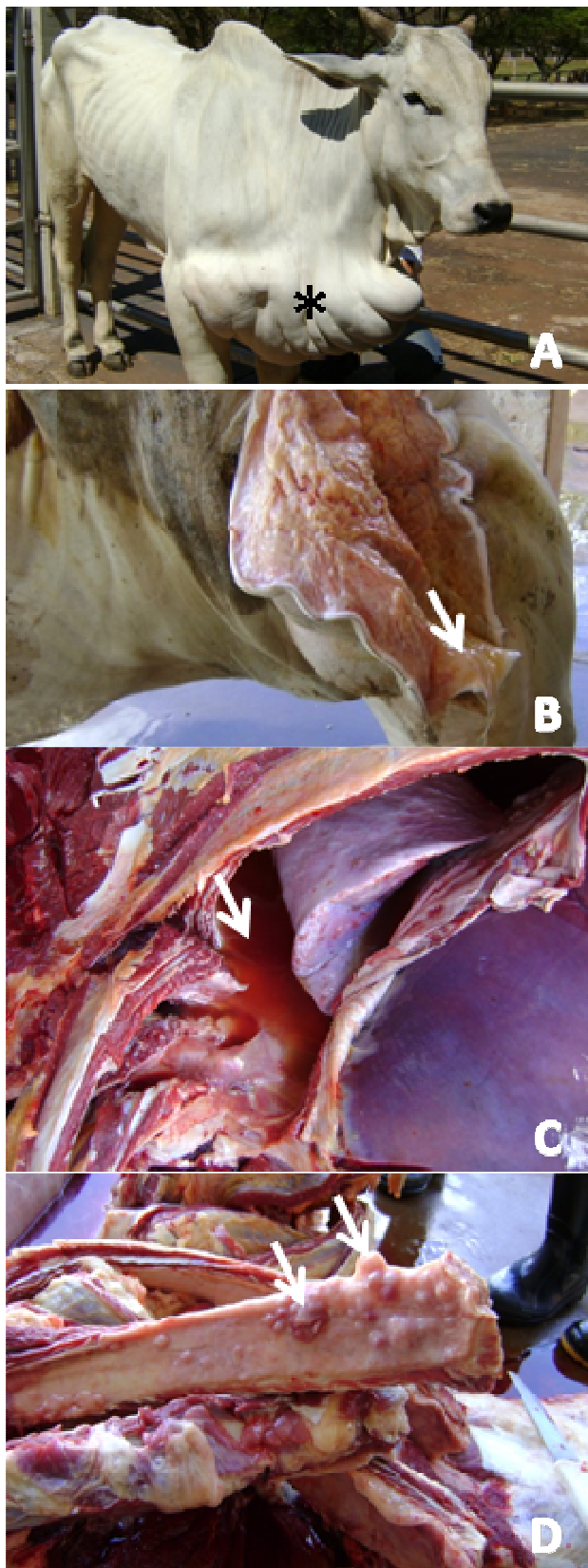
Case Description and Results

A three and a half-year-old Nelore cow presented decreased appetite, weight loss and edema of the chest which was gradually accentuating throughout the pectoral region and dewlap (Figure 1A). Cardiac auscultation revealed tachycardia (90 bpm), with muffling of heart sounds and shallow predominantly abdominal breathing. Leukocytosis (23,900 Leukocytes/mm³) with neutrophilia (67%) and relative lymphocytopenia (27%) was detected on day 45 after the onset of clinical signs, and death ensued 55 days after the onset of clinical signs.

Postmortem evaluation detected subcutaneous edema in the ventral neck (Figure 1B) and pale ocular mucosa. Moderate amount of yellow transudate was found in the abdominal cavity. The liver showed areas of adhesion to the diaphragm and presence of discrete quantity of fibrin on the surface. The thoracic cavity revealed a large amount of serous and bloody fluid (Figure 1C) and several soft nodules adhered to the parietal pleura (Figure 1D) and pericardial sac. On the lung surface yellowish white nodules ranging from 0.5 to 5 cm of diameter (Figure 1E), distributed in all lung lobes were observed. The mediastinal lymph nodes were severely enlarged (Figure 1F). By cytological analysis (Diff-Quick), a predominance of neoplastic lymphocytes, with appearance of immature lymphocytes (lymphoblasts), with marked atypia was verified.

Histological analysis of tumor sections stained with hematoxylin and eosin (HE), revealed neoplastic proliferation of lymphoid cells, which invaded the fatty layer of the pericardium. The neoplastic cells infiltrated the lung and intercostal muscles forming sheets with moderate pleomorphism, anisokaryosis, granular chromatin, hyperchromasia, some kidney-shaped nucleus, and scarce and acidophilic cytoplasm. The presence of cells undergoing apoptosis was marked and mitotic figures were absent (Figure 2).

Immunohistochemical (IHC) analysis of the tumor sections was performed with monoclonal antibodies anti-CD4, anti-CD8 (T lymphocytes marker, Novocastra, NCL-CD4-176 and NCL-CD8-295, respectively) and anti-CD79 (B lymphocytes marker, DAKO/M7051), with



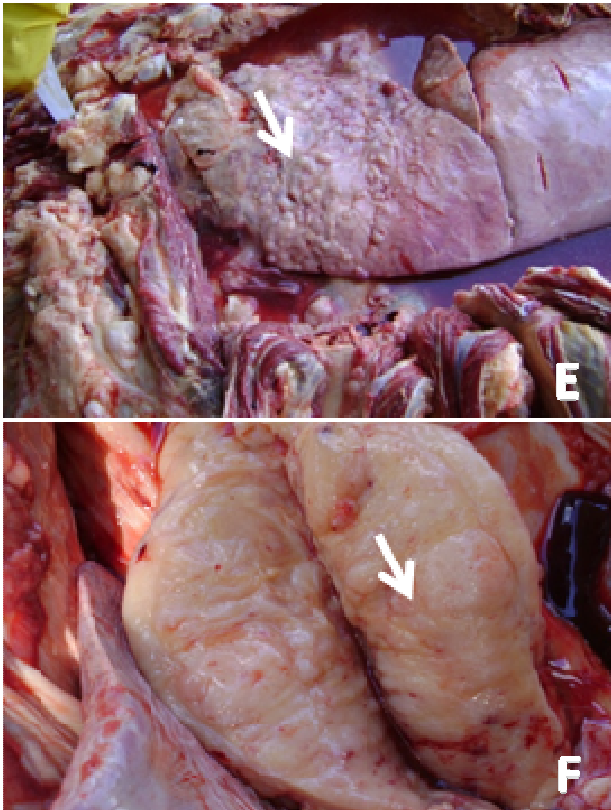


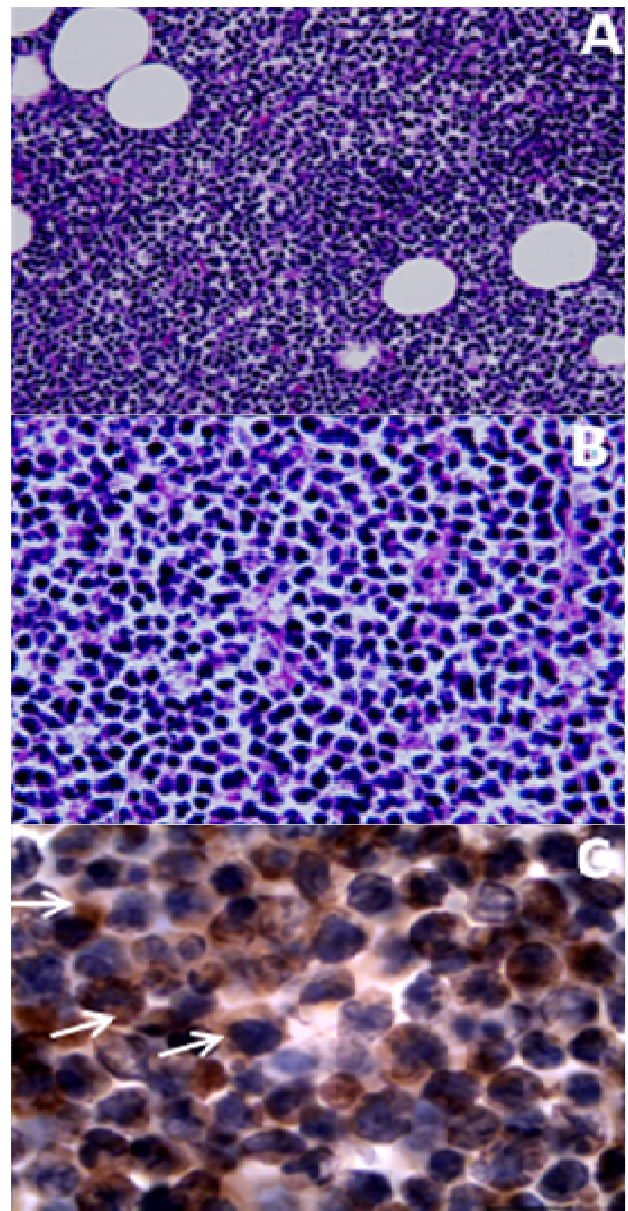
Figure 1 – Nelore cow with thymic lymphoma. (A) Note low body score and swelling (edema) in the pectoral region and dewlap (*). (B) Edema of subcutaneous of the pectoral region and dewlap (arrow). (C) Thoracic region with serous bloody fluid, hydrothorax (arrow). (D) Soft nodules adhered to the parietal pleura and pericardial sac (arrow). (E) Lung with yellowish white nodules on the surface of the organ (arrow). (F) Mediastinal lymph node of a cow with thymic lymphoma. Node swelling without distinction between cortical and medullary layers due to the neoplastic infiltration (arrow)

Streptavidin biotin peroxidase complex (LSAB kit, DAKO/K0690) with diaminobenzine as a chromogen (DAB, DAKO/ K3468) and counterstained with Harris hematoxylin. All antibodies were diluted at 1:50; antigen retrieval was performed by heat (Pascal pressure chamber, DAKO), with a solution of sodium citrate 10 mM (pH 6.0). Endogenous peroxidase was blocked with a 20-minutes incubation in a methanol-8% hydrogen peroxide (30 v/v, Merck). Blocking of unspecific proteins was performed with a commercial product (Protein Block Serum Free, DAKO/ X0909). The CD8 T lymphocyte was the only immunostained cell, with positivity at the cytoplasmic membrane. The CD4 T lymphocyte and B lymphocyte were negative (Figure 2). Positive controls were provided by bovine lymph node sections; negative control tissue sections were incubated with the antibody diluent only.

Discussion

The low body score and edema of the chest and dewlap observed in the Nelore cow are not considered as a pathognomonic clinical sign of thymic lymphoma, because congestive heart failure and traumatic reticulopericarditis, more commonly diagnosed in dairy cattle, present similar clinical signs (12, 14). Trypanosomiasis by *Trypanosoma vivax* and sometimes tuberculosis cause weight loss, lymphadenopathy and edema (1, 12); therefore, these diseases should be considered in the differential diagnosis.

The thymic form of lymphomas is most commonly found in cattle under 30 months of age (8), however in this report the diagnosis was already established in adulthood, which does not rule out the possibility that animals are already carriers of the



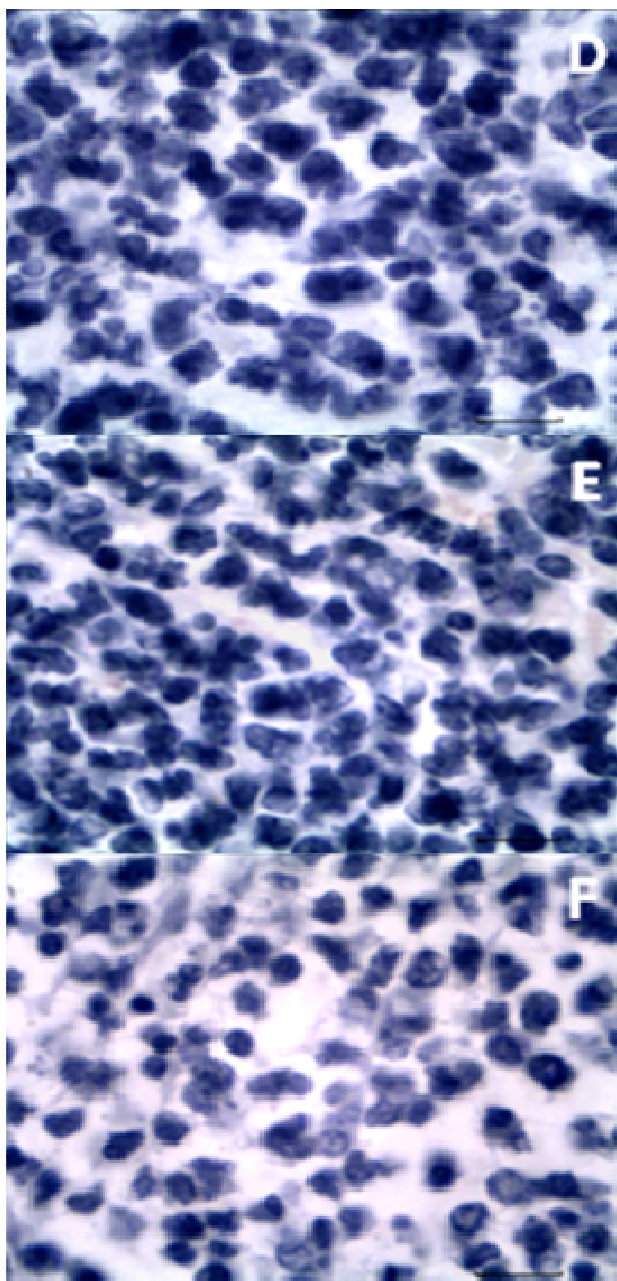


Figure 2 – Photomicrograph of the tumor mass of a Nelore cow with thymic lymphoma. (A) Neoplastic proliferation of lymphoid cells that invade the fatty layer of the pericardium, HE, 100X. (B) HE, 400X. (C) Positive immunostaining of cytoplasmic membranes with anti-CD8 (arrow), Streptavidin Peroxidase Complex, hematoxylin counterstain. 1000X. (D) Negative immunostaining of anti-CD4, Streptavidin Peroxidase Complex, hematoxylin counterstain 1000X. (E) Negative immunostaining of anti-CD79, Streptavidin Peroxidase Complex, hematoxylin counterstain 1000X. (F) Negative control of the immunohistochemical reaction, Streptavidin Peroxidase Complex, hematoxylin counterstain.1000X.

disease from youth with slow evolution and growth of tumor masses, enabling the observation of clinical signs only belatedly.

Animals affected by the thymic form of lymphoma seldom present leukemia, but in advanced cases neoplastic lymphocytes can be detected in the bloodstream without changes in the absolute number of cells (14). In this particular case a relative lymphocytopenia was detected; in absolute values (6.453 lymphocytes/mm³) the numbers were between normal parameters (2500 – 7500 lymphocytes/mm³) (12), corroborating findings by VALLI and PERRY (14). Neutrophils were above the normal range 600 – 4000 neutrophils/mm³ (12).

Tumor infiltrates were identified in the lungs, visceral and parietal pleura, associated with copious amounts of fluid in the thoracic cavity. Thymic lymphomas can infiltrate surrounding tissues, compressing organs and blood vessels triggering hydrothorax (14) thus probably the edema of the chest and dewlap arises from the difficulties of venous return associated with venous compression.

In the immunophenotyping of the reported tumor was found a predominance of CD8 T cell; although the presumptive diagnosis of thymic lymphoma can be performed by cytology, it is not possible to classify the tumor in a B or T cell origin (14). YAMAZAKI et al. (15) marking thymic lymphoma tumor cells of a 14-month-old Holstein steer with CD3 polyclonal antibodies did not determine the subtype of T lymphocytes. KAGAWA et al. (6) e MURAYAMA et al. (9) showed that B lymphocytes were the predominant cells in thymic lymphoma in cattle aged between 216 days and 20 months, using anti-CD79a antibody (Dako and WC1-N3 - Veterinary Medical Research and Development, Pullman, WA, USA).

Conclusion

Gross and microscopic findings were consistent with the diagnosis of thymic lymphoma in the cow of this report. Immunophenotyping of the tumor showed predominance of CD8 T lymphocytes. To the authors' knowledge this is the first report of immunophenotyping of thymic lymphoma in Nelore cattle.

Acknowledgements

We would like to thank Carolina Aralda and Lorena Diaz for their excellent technical help. This work was supported by grants from Fondo para la Investigación Científica y Tecnológica 05-33987, Secretaría de Ciencia y Técnica, Universidad Nacional de La Plata, and Cabaña Argentina SA, Argentina.

References

1. CADIOLI FA., BARNABÉ PA., MACHADO RZ., TEIXEIRA MCA., ANDRÉ MR., SAMPAIO PH., FIDELIS JUNIOR OL.,

- TEIXEIRA MMG., MARQUES LC. First report of Trypanosoma vivax outbreak in dairy cattle in São Paulo State, Brazil. **Bras. J. Vet. Parasitol.**, 2012, 21,2, in press.
2. DUNCAN JUNIOR RB., SCARRATT WK, BUEHRING GC. Detection of bovine leukemia virus by in situ polymerase chain reaction in tissues from a heifer diagnosed with sporadic thymic lymphosarcoma. **J. Vet. Diagn. Invest.**, 2005, 17, 190–194.
 3. HATFIELD CE., REBHUN WC., DILL SG. Thymic lymphosarcoma in three heifers. **J. Am. Vet. Med. Ass.**, 1986, 189, 1598–1599.
 4. HOLMES LA., SCOTT PR., ALDRIDGE BM. Thymic lymphosarcoma with metastases causing spinal cord compression and pelvic limb paresis in a heifer. **Brit. Vet. J.**, 1990, 146, 91–92.
 5. JACCOBS RM., MESSICK JB., VALLI VE. Tumor of the skin hemolymphatic system. In: MEUTEN DJ. Ed. **Tumors in domestic animals**. 4. ed., Ames: Iowa State Press. Chap. 3, 119–198, 2002.
 6. KAGAWA Y., TOMITA K., NAKATANI H., SATO K., WADA Y., ISHIKAWA Y., KADOTA K. Immunohistochemical characterization of five types of lymphoid neoplasms in calves. **Jpn. Agr. Res. Q.**, 2009, 43, 239-245.
 7. MATTHEWS HK., HUNT E., DUNCAN DE. Thymic and mammary lymphosarcoma in a three-year-old heifer. **J. Am. Vet. Med. Ass.**, 1992, 200, 699–701.
 8. MOULTON JE., HARVEY JW. Tumors of the lymphoid and hematopoietic tissues. MOULTON JE. **Tumors in domestic animals**. 3. ed. London: University of California Press. Chap. 6, 231-307, 1990.
 9. MURAYAMA S., SATO K., IKEHATA T., WADA Y., ISHIKAWA Y., KADOTA K. Cytologic and immunophenotypic investigation of lymphohematopoietic neoplasms in cattle. **Jpn. Agr. Res. Q.** 2011. 45, 225 – 231.
 10. NASIR, KS. Sporadic juvenile thymic lymphoma in a 6-month-old Holstein heifer. **Can. Vet. J.**, 2005, 46, 831-833.
 11. PARODI AL., DACOSTA B., DJILALI S., MICHEL B., ALOGNINUWA TH., FEMENIA F., CRESPEAU F., FONTAINE JJ., THIBIER M. Preliminary report of familial thymic lymphosarcoma in Holstein calves. **Vet. Rec.**, 1989, 125, 350–353.
 12. RADOSTITS OM., GAY CC., BLOOD DC., HINCHLIFF KW. Diseases of the spleen, lymphadenopathy and thymic disease. In: **Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses**, 9. ed. London: Saunders, 2000, 417– 420.
 13. SMITH HA. The Pathology of Malignant Lymphoma in Cattle: A Study of 1113 Cases. **Vet. Pathol.** 1965, 2, 68-94.
 14. VALLI VEO., PARRY BW. The hematopoietic system. In: JUBB KVF., KENNEDY PC., PALMER N. Eds. **Pathology of domestic animals**. 4. ed. San Diego: Academic Press, 1993. Vol. 3, chap 2, 101-266.
 15. YAMAZAKI Y., ISHIKAWA Y., SHIBAHARA T., KADOTA K., ISHINO S. An Immunohistochemical and Ultrastructural Study of Thymic Lymphoma in a Steer. **Jpn. Agr. Res. Q.**, 2000, 34, 195-198.