

Multicentric T-Cell Lymphoma in a Creole filly

Linfoma multicêntrico de Células T em uma potra Crioula

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Highlights:

Macroscopic and microscopic findings were compatible with multicentric lymphoma.

The histopathological evaluation confirmed the diagnosis of multicentric lymphoma.

The immunohistochemistry assessment revealed neoplastic origin in the T lymphocytes.

Abstract

A 2-year-old creole filly was referred to us for evaluation because of the clinical suspicion of infection by *Streptococcus equi*. It presented with progressive weight loss and increased volume of the submandibular, retropharyngeal, and precrural lymph nodes. General clinical examinations and laboratory tests revealed dehydration, anemia, leukopenia, hyperfibrinogenemia, and thrombocytopenia. The initial treatment for equine adenitis did not achieve significant results, and new hematological and biochemical tests and lymph node cytology by puncture were performed. Cytology revealed cells compatible with neoplastic lymphocytes, resulting in the suspicion of lymphoma. The animal died from general weakness and was sent for autopsy. Macroscopically, generalized lymphadenomegaly and splenomegaly were observed, with multiple nodules and tumor lesions in the splenic parenchyma. There was an irregular nodule in the medullary layer of the right kidney. The liver and lungs were slightly enlarged, with petechiae and multifocal suffusions. Histopathological evaluation of different organ specimens revealed intense proliferation of the neoplastic lymphoid cells, invading the adjacent tissues, with moderate cellular pleomorphism. Immunohistochemistry of the lymph node sections with neoplastic infiltration revealed multicentric T-cell lymphoma. In horses, cases of lymphomas are rare and should be differentiated from other causes that induce lymphadenomegaly in this species.

Key words: Horse. Lymphadenopathy. Neoplasia. T lymphocytes.

Resumo

Uma potra da raça crioula, com dois anos de idade foi encaminhada para avaliação clínica com suspeita de infecção por *Streptococcus equi*. A paciente apresentava emagrecimento progressivo e aumento dos linfonodos submandibulares, retrofaringeos e pré-cruais. A potra foi submetida a exames clínicos gerais e de laboratório, apresentando desidratação, anemia, leucopenia, hiperfibrinogenemia e

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trombocitopenia. O tratamento inicial para adenite equina não obteve resultados significativos, e novos exames hematológicos, bioquímicos e citologia da punção de linfonodos foram realizados. O resultado da citologia revelou células compatíveis com linfócitos neoplásicos, o que levou a suspeita de linfoma. O animal morreu em consequência da debilidade de seu estado geral e foi encaminhado para necropsia. Macroscopicamente, observou-se linfadenomegalia generalizada, esplenomegalia com múltiplos nódulos no parênquima do baço, bem como lesões tumorais caracterizadas por rim direito apresentando nódulo irregular na camada medular, fígado levemente aumentado e pulmões com petéquias e sufusões multifocais. Amostras de variados órgãos foram submetidos a avaliação histopatológica, a qual revelou intensa proliferação de células linfoides neoplásicas com invasão de tecidos adjacentes com moderado pleomorfismo celular. Secções de linfonodos com infiltrado neoplásico foram submetidas à avaliação imuno-histoquímica (IHQ) a qual determinou tratar-se de linfoma de células T multicêntrico. Em equinos, esse tipo de neoplasma é pouco frequente e deve ser diferenciado de outras causas que induzem linfadenomegalia nessa espécie.

Palavras-chave: Equino. Linfadenopatia. Neoplasia. Linfócitos T.

Introduction

Lymphomas are heterogeneous hematopoietic neoplasms, with various clinical presentations and laboratory and morphological changes (Meyer, Delay, & Bienzle, 2006; Gravena et al., 2018). Lymphomas are uncommon in horses and have no sex, race, or age predilections (Meyer et al., 2006). Their clinicopathological presentations in different domestic species have been classified into thymic, alimentary, multicentric, cutaneous (Gravena et al., 2018) and solitary extragonadal forms (Taintor & Schleis, 2011).

Clinical symptoms observed in the initial developmental phase of lymphomas are non-specific, such as lethargy, weight loss, and depression, and its definitive diagnosis requires histopathological examination (Taintor & Schleis, 2011). Further, the prognostication tends to be delayed (Gravena et al., 2018). In such cases, the laboratory findings vary, although they present with hematologic evidence of the disease, such as unresponsive anemia, thrombocytopenia, leukopenia, and hyperfibrinogenemia (Meyer et al., 2006). Variations in the tumoral behavior hinders the definitive diagnosis, thus requiring a detailed clinical-pathological evaluation. Herein, we report the clinicopathological aspects of a case of multicentric lymphoma in a creole filly.

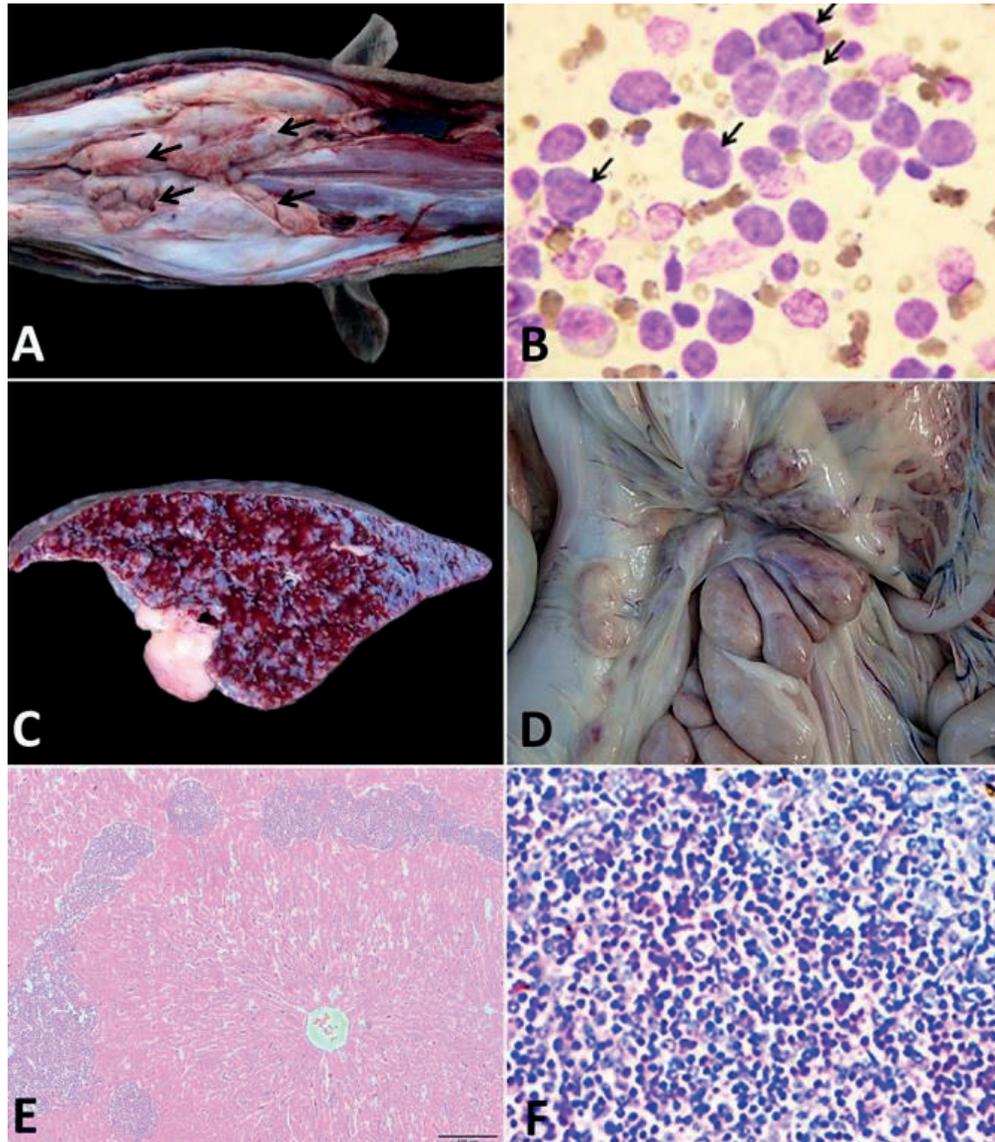
Case Report

A 2-year-old creole filly was referred to us for clinical care, with the suspicion of chronic infection by *Streptococcus equi*, presenting with clinical signs of productive cough, febrile episodes, mucopurulent nasal discharge, and increased volume of the submandibular, retropharyngeal (Figure 1A), and precucular lymph nodes. The clinical assessment revealed moderate dehydration, a heart rate of 66 beats/min, a respiratory rate of 24 breaths/min, and rectal temperature of 40°C.

Fluid therapy with 0.9% sodium chloride, flunixin meglumine therapy, and penicillin-based antibiotic therapy were provided for approximately 1 month as the initial treatment. The penicillin-based antibiotic therapy was suspended after the animal showed no clinical improvements. Warm compresses and topical rubefaciants were applied to reduce the volume and drain the submandibular lymph nodes. The hematological examination during hospitalization revealed clinical anemia, thrombocytopenia, neutropenia, and slight elevation in fibrinogen, while the biochemical test results were within the normal range. On hospitalization day 6, the filly received blood transfusion because of persistent anemia and thrombocytopenia, and an improvement in presentations of anemia and hyperfibrinogenemia was observed. Subsequently, the filly presented with progressive depression and

ataxia, which were unresponsive to the administered therapies. Based on the clinical history, laboratory results, and clinical findings, neoplasia was suspected, which was confirmed with fine-needle aspiration cytology of the submandibular lymph nodes revealing many round cells with scarce

cytoplasm and small and large centroblastic cells with moderate anisocytosis and anisokaryosis, indicating a diagnosis of lymphoma (Figure 1B). Due to worsening of the clinical condition, the animal died about 40 days after the onset of clinical symptoms and was transferred for necropsy.



A. The submandibular region showing diffusely enlarged submandibular and retropharyngeal lymph nodes. B. Lymph node aspiration cytology characterized by large and small centroblastic neoplastic lymphoid cells with scarce cytoplasm and pleomorphic nucleus with moderate anisocytosis and anisokaryosis (arrows; Fast Panoptic; Obj. 40 \times). C. The transverse section of the spleen with multiple pale neoplastic nodules in the parenchyma, indicating the follicular pattern. D. Multiple sharply enlarged mesenteric lymph nodes. E. Intense infiltration of the neoplastic lymphoid cells into the periportal space of the liver (hematoxylin and eosin; bar = 100 μ m). F. The lymph node section with neoplastic nodules presenting as neoplastic lymphocytes with moderate cytoplasmic immunostaining by anti-CD3 antibody, characteristic of T lymphocytes (immunohistochemistry with streptavidin and alkaline phosphatase; anti-CD3 antibody; Obj. 10 \times).

Figure 1. Multicentric lymphosarcoma in a foal.

Macroscopic evaluation showed bilateral enlargement of the ventro-caudal aspect of the mandible with diffuse marked lymph node enlargement. The superficial lymph nodes were soft to cut because of the loss of architecture and were found infiltrated by multiple whitish neoplastic nodules, which were interspersed with dark-red areas. Moderate splenomegaly with marked capsular irregularity was observed, which was verified by slicing the multiple whitish stained proliferated nodules interspersed in the parenchyma (Figure 1C). There was a single irregular dark-red nodule, measuring approximately 1.0 cm in diameter, in the medullary layer and protruding into the cortex of the right kidney, with a background of gray area. In the intestines, slight mucosal thickening was noted, with marked evidence of rare Peyer's patches and mucosal focal ulcers and marked enlargement of the mesenteric lymph nodes (Figure 1D). The liver was slightly enlarged and showed accentuation of the lobular pattern. The lungs were armed and shiny and contained petechiae and multifocal suffusions on the surface.

Samples of different organs and tissues were collected, fixed in 10% formalin, histologically processed, and stained with hematoxylin and eosin. Microscopically, the nodules located in different organs comprised of intense proliferation of neoplastic lymphocytes. The lymph nodes presented with loss of architecture, without distinctions of the medullary and cortical layers or lymphoid follicles. The neoplastic cells were large, with loose chromatin and prominent nucleolus, which was usually centered in the nucleus. Up to four mitoses were observed per high-magnification field (40×). Neoplastic lymphocytic proliferation was observed in the liver, especially in the periportal areas (Figure 1E). No alterations were observed in the sections of the bone marrow collected from the sternum. Histological sections with predominance of neoplastic cells of the lymph nodes were sent for immunohistochemistry (IHC) assessment, based on the alkaline phosphatase-linked streptavidin

complex method, and revealed with Permanent Red, using anti-CD3 antibody (Dako A0452) at a dilution of 1:200. A second IHC method was employed, using the peroxidase-linked streptavidin complex and developed with DAB, in which CD79 alpha (Dako M7050) antibodies at 1:10 dilution and CD68 (Dako MO718) at 1:100 dilution were used. IHC evaluation of the sections revealed moderate multifocal cytoplasmic CD3 labelling in the cytoplasm (Dako A0452), indicating neoplastic origin in the T lymphocytes (Figure 1F).

Discussion

Owing to the initial clinical findings of productive cough, febrile episodes, mucopurulent nasal discharge, and increased volume of the submandibular, retropharyngeal, and precrural lymph nodes, equine adenitis, caused by *Streptococcus equi*, was suspected (Moraes, Vargas, Leite, Nogueira, & Turnes, 2009). An antibiotic therapy was provided, which showed no response, thus resulting in the suspension of therapy and rejection of this clinical suspicion. Cases of multicentric lymphoma with a history of respiratory signs similar to this case have been reported (Campos et al., 2014; Gravena et al., 2018). Therefore, neoplasm should be considered in the differential diagnosis of cases presenting with similar clinical signs. In horses, the multicentric form of lymphoma seems to be the most common, with involvement of the lymph nodes and thoracic and abdominal organs (Durham, Pillitteri, San Myint, & Valli, 2013), as observed in this case.

Hematological examination during hospitalization revealed anemia, thrombocytopenia, neutropenia, and hyperfibrinogenemia, which are often reported in cases of equine lymphosarcoma (Meyer et al., 2006; Gravena et al., 2018). Animals with lymphoma typically develop unresponsive anemia, as described by Meyer et al. (2006). Anemia is more common and often associated with the agglutination of erythrocytes while hyperglobulinemia may be caused by the

premature destruction of erythrocytes by antibodies or inadequate production of erythrocytes due to myeloptysis or both. Therefore, immune-mediated hemolytic anemia or thrombocytopenia and mild elevation in the serum enzymes due to hepatic injury are commonly observed (Morris, 2000). The present equine species showed pancytopenia, which suggested myeloptysis (Meyer et al., 2006); however, evaluation of the bone marrow samples from the sternum revealed no significant changes. The assessment of the bone marrow may be insufficient to observe neoplastic infiltration with myeloid tissue destruction, commonly observed in these cases (Fry & McGavin, 2009). However, the involvement of the bone marrow with development of lymphocytic leukemia is uncommon in horses with multicentric lymphoma, although it can occur in cases of extensive damage to the myeloid lineage (Taintor & Schleis, 2011), which could not be verified in this case, possibly due to the collection of insufficient material, which suggests the need for many bone marrow samples to be evaluated in cases of lymphoma.

Diffuse lymphadenomegaly, appearance of the lymph nodes upon slicing of the neoplastic nodules, and splenomegaly, together with marked capsular irregularity and multiple nodules in the splenic parenchyma, were compatible with multicentric lymphoma, which was confirmed with the histopathological evaluation showing the lymph nodes with large neoplastic cells, containing up to four mitoses per high-amplification field. These findings confirmed the diagnosis of multicentric lymphoma. Histologically, the lymphomas are characterized by a monomorphic population of atypical lymphocytes, well-differentiated or not, depending on the degree of malignancy (Fry & McGavin, 2009). In general, the atypical lymphocytes present as large cells at variable proportions between the nucleus and the cytoplasm, with multiple nucleoli, nuclear achromatic agglomeration, cytoplasmic basophilia, vacuolization, mitotic figures, and binucleated cells (Morris, 2000).

The definitive diagnosis of lymphoma should be made through histopathological examination and/or fine-needle aspiration cytology. Complementary examinations, such as IHC and immunophenotyping, can be used to identify the cellular origin of the neoplasm. In humans, lymphomas are classified into Hodgkin's, characterized by the presence of giant cells, which are usually derived from B lymphocytes, and non-Hodgkin's lymphoma, without the presence of giant cells (Taintor & Schleis, 2011). In animals, the classification of lymphomas is hampered by the lack of specific markers for the species (Campos et al., 2014). However, lymphomas in animals may be classified based on the Revised European American Lymphoma classification system, which considers the cellular type: B precursors or mature and precursor T or null (Valli, 2007).

As for the IHC assessment, moderate multifocal cytoplasm CD3 staining (Dako A0452) revealed a neoplastic origin in the T lymphocytes. Meyer et al. (2006) reported that most cases of lymphoma diagnosed in equine slaughterhouse samples had originated from the T cells. Approximately half the cases of neoplasms originating from the B lymphocytes are B-cell lymphomas rich in T lymphocytes. Morrison, Freil, Henderson, Hahn and Smith (2008) reported a case of proliferative disease of the central nervous system with characteristics of lymphoma in a horse, in which the disease could not be accurately diagnosed using IHC. However, Lehmecker et al. (2014) used IHC to detect T cells in the lymph nodes, intestines, and nerves in three horses with manifestations of lymphoma with neoplastic infiltration in the nerves and spinal nerve roots. In the present report, IHC labelling alone could confirm the T-cell origin.

In this case report, the routine inspection of the spinal cord was fully performed at autopsy, with no evaluations of macroscopic or microscopic changes in the segments. In another report, one horse diagnosed with multicentric lymphoma of T cells presented with ataxia due to the extension of lymphoma in the epidural space from the proximo-

cervical region up to the mid-thoracic region, compressing the spinal cord from the first cervical vertebra to the seventh thoracic vertebra, which did not occur in the present case (Ueno, Wada, Mashita, Kuwano, & Katayama, 2012).

Conclusion

Anamnesis, clinical examination, and detailed complementary tests, with evaluation of multiple components, are fundamental in cases of suspected diagnosis of multicentric lymphoma. In this case, the treatment for respiratory disease, which was suspected initially, was ineffective; however, with the cytopathological examination, neoplasia was suspected, which may help minimize the costs of possible ineffective treatments and the patient's suffering caused by therapeutic misconduct.

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References

- Campos, C. G. de, Lopes, L. L., Silva, G. S. e, Cruz, R. A. S. da, Falsoni, P. C. G., Rondelli, A. L. H.,... Pescador, C. A. (2014). Linfoma de células T multicêntrico em um equino quarto de milha. *Acta Scientiae Veterinariae*, 42(1), 40.
- Durham, A. C., Pillitteri, C. A., San Myint, M., & Valli, V. E. (2013). Two hundred three cases of equine lymphoma classified according to the world health organization (WHO) classification criteria. *Veterinary Pathology*, 50(1), 86-93. doi: 10.1177/0300985812451603.
- Fry, M. M., & McGavin, M. D. (2009). Medula óssea células sanguíneas e sistema linfático. In M. D. McGavin, J. F. Zachary, *Bases da patologia em veterinária* (pp. 798-800). Rio de Janeiro: Elsevier.
- Gravena, K., Rivera, G. G., Navarrete, R. A., Jark, P. C., Camacho, A. A., & Lacerda, J. C., Neto. (2018). Linfoma mediastinal em equino - relato de caso. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 70(1), 57-63. doi: 10.1590/1678-4162-9424.
- Lehmbecker, A., Liebing, J., Barthel, Y., Habierski, A., Cavalleri, J., Puff, C.,... Beineke, A. (2014). Neurolymphomatosis in three horses with multicentric t-cell-rich b-cell lymphoma. *Journal of Comparative Pathology*, 151(2-5), 181-185. doi: 10.1016/j.jcpa.2014.04.004.
- Meyer, J., Delay, J., & Bienzle, D. (2006). Clinical, laboratory, and histopathologic features of equine lymphoma. *Veterinary Pathology*, 43(6), 914-924. doi: 10.1354/vp.43-6-914.
- Moraes, C. M. de, Vargas, A. P. C., Leite, F. P. L., Nogueira, C. E. W., & Turnes, C. G. (2009). Adenite equina, sua etiologia, diagnóstico e controle. *Ciência Rural*, 39(6), 1944-1952. doi: 10.1590/S0103-84782009000600050.
- Morris, D. D. (2000). Doenças do sistema hemolinfático. In S. M. Reed, W. M. Bayly, *Medicina interna equina* (pp. 495 e 496). Rio de Janeiro: Guanabara Koogan.
- Morrison, L. R., Freel, K., Henderson, I., Hahn, C., & Smith, S. H. (2008). Lymphoproliferative disease with features of lymphoma in the central nervous system of a horse. *Journal of Comparative Pathology*, 139(4), 256-261. doi: 10.1016/j.jcpa.2008.07.008.
- Taintor, J., & Schleis, S. (2011). Equine lymphoma. *Equine Veterinary Education*, 23(4), 205-213. doi: 10.1111/j.2042-3292.2010.00200.x.
- Ueno, T., Wada, S., Mashita, S., Kuwano, A., & Katayama, Y. (2012). Pathological findings in a case of equine T-cell lymphoma associated with ataxia. *Journal of Equine Veterinary Science*, 32(6), 315-319. doi: 10.1016/j.jevs.2011.11.012.
- Valli, V. E. O. (2007). Hematopoietic system. In M. G. Maxie (Ed.), *Jubb, Kennedy, and Palmer's pathology of domestic animals* (5nd ed., Vol. 3, pp. 107-324). Philadelphia: Saunders Elsevier.