



Extranodal lymphoma in a bush dog (*Speothos venaticus*) - case report

[*Linfoma extranodal em um cachorro-do-mato-vinagre (Speothos venaticus) - relato de caso*]

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ABSTRACT

A captive 7-year-old male bush dog (*Speothos venaticus*) was diagnosed with lymphoma affecting the kidneys, adrenal glands, liver, and spleen. The animal developed renal failure and was euthanized due to poor prognosis. Grossly, both kidneys were enlarged with multiple nodules. Histologically, the neoplasm was an infiltrative and poorly demarcated round cell tumor. Two morphologically distinct cell populations were observed, smaller cells with a lymphocytic morphology, and another population of larger and pleomorphic cells. Most of the smaller cell population, approximately 40% of the population within the neoplasm, were CD3 positive. Neoplastic cells were CD45, CD11d, and granzyme B positive, and negative for CD20, CD79a, PAX5, CD163, and myeloperoxidase. This is the first reported case of lymphoma in a bush dog. This report demonstrated the suitability of several cell surface markers for differential diagnosis of round cell tumors in this species.

Keywords: lymphoma, immunohistochemistry, neoplasm, wildlife

RESUMO

Um cachorro-do-mato-vinagre (Speothos venaticus), de sete anos de idade, mantido em cativeiro, foi diagnosticado com linfoma que havia afetado os rins, as adrenais, o fígado e o baço. O animal desenvolveu insuficiência renal e foi submetido à eutanásia devido ao prognóstico desfavorável. Macroscopicamente, ambos os rins estavam aumentados de tamanho, com múltiplos nódulos. Histologicamente, a neoplasia era infiltrativa, pobremente delimitada e constituída por células redondas. Duas populações distintas foram observadas: células pequenas com morfologia linfocítica e células grandes e pleomórficas. A maior parte da população de células pequenas, correspondendo a aproximadamente 40% da população celular na neoplasia, foi positiva para CD3. As células neoplásicas foram positivas para CD45, CD11d e granzima B e negativas para CD20, CD79a, PAX5, CD163 e mieloperoxidase. Este é o primeiro caso de linfoma em um cachorro-do-mato-vinagre. Tal relato demonstra a utilidade de vários marcadores de superfície celular para o diagnóstico diferencial de tumores de células redondas nessa espécie.

Palavras-chave: linfoma, imuno-histoquímica, neoplasma, animal silvestre

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INTRODUCTION

Lymphoma is the most common neoplasm of the hemolymphatic system in domestic dogs and cats. Lymphomas may develop in any organ, although it affects lymphoid organs such as lymph nodes, spleen, and liver with higher frequency (Vail *et al.*, 2013). Multicentric manifestation is the most common form of lymphoma in dogs, corresponding to approximately 75% of all cases. Classification of canine lymphoma is based on clinical signs, cellular morphology, and immunophenotyping (Vail *et al.*, 2013). The incidence of canine as well as human lymphoma has increased over the past decades so a possible exposure to predisposing factors must be considered (Pastor *et al.*, 2009).

The bush dog is a small canid that occurs in South America and is considered an endangered species. Its distribution includes the Brazilian states of Goiás, São Paulo, Paraná, Pará, Tocantins, Maranhão, Mato Grosso, and Mato Grosso do Sul as well as the entire Amazon region. The goal of this report was to describe histopathological and immunohistochemical features of an extranodal lymphoma in a captive bush dog (*Speothos venaticus*).

CASE REPORT

A 7-year-old male captive bush dog (*Speothos venaticus*) housed at the Fundação Zoo-Botânica de Belo Horizonte (Brazil) had a diagnosis of *Leishmania infantum* (synonym *L. chagasi*) infection by xenodiagnosis in 2013. In March 2015, the dog was admitted to the zoo veterinary hospital presenting with vomiting and diarrhea, prostration, and anorexia. Blood chemistry analysis indicated elevated concentrations of creatinine (7.0mg/dL; reference values: 0.5 – 1.5mg/dL) and blood urea nitrogen (266mg/dL; reference: 15 – 50mg/dL) as well as mild hypoproteinemia (6.6g/dL; reference: 7.0 – 8.5g/dL) with an albumin/globulin ratio of 0.5 (albumin: 2.2g/dL; globulin: 4.4g/dL). Hematologic analysis indicated thrombocytopenia (130,000 platelets/ μ L; reference: 200,000 – 570,000). Based on clinical signs and laboratory results, a presumptive diagnosis of renal failure was established. Due to the poor clinical condition

and unfavorable prognosis the animal was euthanized and subjected to necropsy.

Grossly, the animal was dehydrated and had mildly icteric mucosa. There was marked dental tartar with multifocal ulcerative gingivitis and glossitis. The animal had multiple pressure sores on the skin of the ventral thorax and adjacent to lateral osseous prominences. The kidneys were moderately enlarged with multiple nodules appearing on the surface of the organ as well as on the cut surface (Figure 1). The spleen and the liver were moderately enlarged, and the liver had a mild and diffuse yellow discoloration. There were petechiae on the urinary bladder mucosa, and multiple small ulcerative lesions in the mucosa of the stomach.



Figure 1. Kidney, lymphoma in a bush dog (*Speothos venaticus*). Top: left and right kidneys with nodules protruding on the organ surface. Bottom: cut surface of nodular lesions with a whitish solid tissue with extensive areas of hemorrhage.

Histologically, a round cell neoplasm was observed in the kidney, adrenal gland, spleen,

and liver. The neoplasm was densely cellular, infiltrative, non-encapsulated, and replaced extensive areas of the normal tissues in these organs (Figure 2). The neoplasm was composed of round cells with well-defined cell margins and arranged in solid sheets. Large neoplastic cells contained abundant lightly eosinophilic homogeneous cytoplasm, and nuclei were large, varying from 7-10 μ m in diameter, pleomorphic with occasional indentations, vesicular chromatin, and one to two prominent nucleoli. Anisocytosis and anisokaryosis were marked, and rare binucleated cells were observed. Mitotic activity varied among different sites, with averages of 8 mitotic figures per 40x field in the kidney, 6, 2 or rare mitotic figures in the adrenal gland, spleen and liver, respectively. There were a few atypical mitotic figures and vascular invasion by neoplastic cells. Admixed with the neoplastic population, there were large numbers of well differentiated small lymphocytes, which in some areas encompassed approximately 40% of the entire population. In the remaining renal parenchyma, there were changes compatible with a diffuse and severe membranous glomerulopathy. Numerous megakaryocytes were observed in the spleen, characterizing extramedullary thrombopoiesis, and small amounts of eosinophilic, amorphous, and acellular proteinaceous material compatible with amyloid. There was moderate and diffuse hepatic lipidosis. In the lung, there was a mild to moderate interstitial inflammatory cell infiltrate composed mostly of histiocytes, with some lymphocytes and neutrophils and rare plasma cells; and mild anthracosis. There was mild testicular degeneration. The heart, small and large intestines, and pancreas had no histological changes.

The neoplastic cells were weakly positively for CD45, and strongly positive for granzyme B and CD11d, and negative for CD20, CD79a, PAX5, CD163 (not shown), and myeloperoxidase (Figure 3). A few neoplastic cells and the population of small lymphocytes were positive for CD3. No metachromatic granules were observed in the cytoplasm of neoplastic cells

with Giemsa staining. Approximately 30% of the cells infiltrating the neoplasm were positive for Iba1, with a few intravascular Iba-1 positive cells. A few CD20 positive cells, that were interpreted as pre-existing B cells with no neoplastic morphology, were observed. Table 1 describes the immunohistochemistry markers employed in this case.

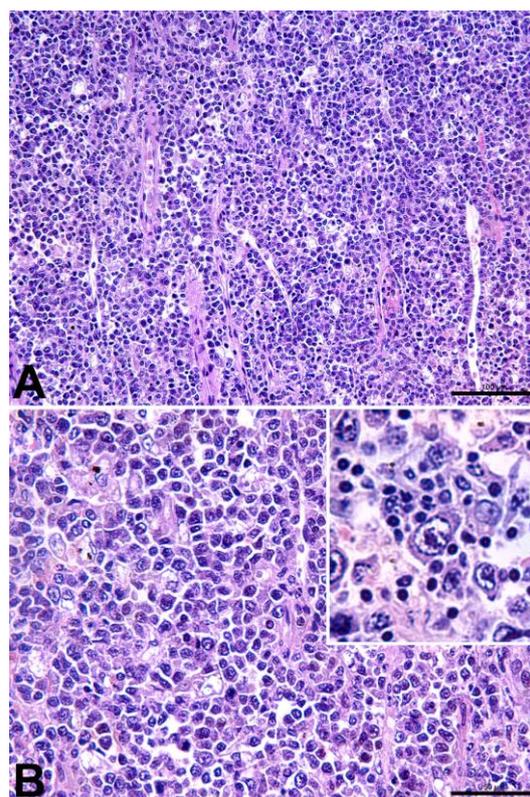


Figure 2. Kidney, lymphoma in a bush dog (*Speothos venaticus*). (A) densely cellular and infiltrative round cell tumor HE, Bar = 100 μ m; (B) Solid sheets of neoplastic cells characterized by larger and pleomorphic cells, with abundant eosinophilic cytoplasm and marked anisokaryosis, infiltrated with abundant smaller cells with scant cytoplasm and central round nuclei that resemble well differentiated lymphocytes. HE, Bar = 50 μ m.

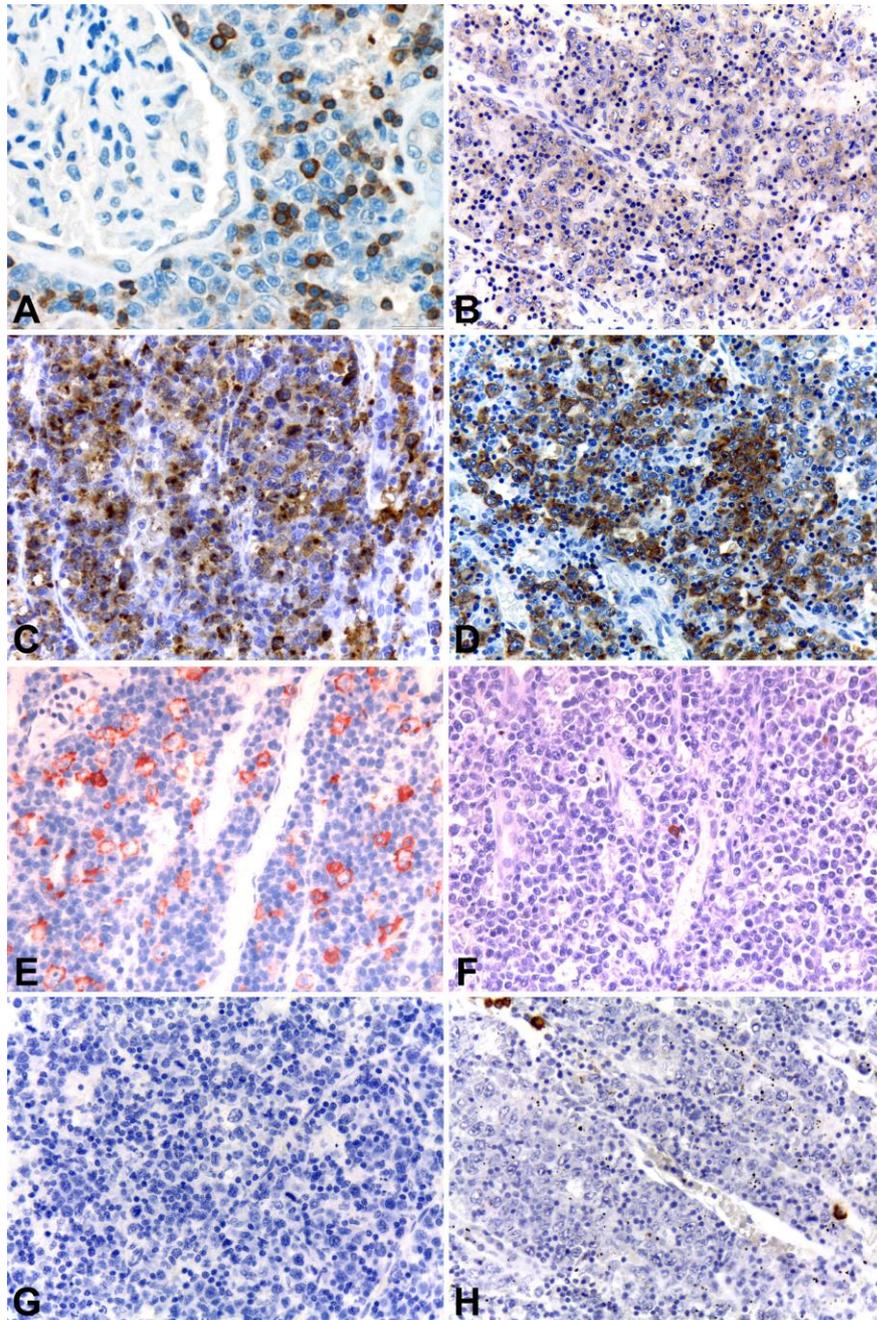


Figure 3. Kidney, lymphoma in a bush dog (*Speothos venaticus*). Immunohistochemistry panel: (A) CD3 positive cells – mostly smaller cells with lymphocyte morphology, corresponding to approximately 40% of the cell population within the neoplasm; (B) CD45 – the population of large and pleomorphic neoplastic cells stained weakly but homogeneously for CD45; (C) Granzyme B – pleomorphic neoplastic cells stained strongly for granzyme B; (D) CD11d – pleomorphic neoplastic cells stained strongly for CD11d; (E) Iba1 – there was a considerable number of Iba1 positive cells with a histiocytic morphology scattered within the neoplasm. Neoplastic cells were negative for CD79a (F), PAX5 (G), and myeloperoxidase (H), with a few positive pre-existing non neoplastic cells that served as internal controls. IHC; the chromogen was 3,3-diaminobenzidine tetrachloride (DAB) for all sections, except Iba1 whose chromogen was 3-amino-9-ethylcarbazole (AEC). Sections were counterstained with Mayer's hematoxylin.

Table 1. Immunohistochemistry markers employed in this case

Marker	Type	Clone or catalogue number	Dilution	Supplier
CD3	Monoclonal	SP7	1:150	Fisher, Waltham, MA 02451, USA.
CD11d	Monoclonal	CA12.10C12	1:50	Dr. P. Moore, UC-Davis, Davis, CA 95616, USA.
CD20	Polyclonal, rabbit	Cat# E2560	1:1,000	Spring Biosciences, Pleasanton, CA 94566, USA.
CD45	Monoclonal	CA12.10C12	1:20	Dr. P. Moore, UC-Davis, Davis, CA 95616, USA.
CD79a	Monoclonal	HM47/A9	1:50	Biocare Medical, Pacheco, CA 94553, USA.
CD163	Polyclonal, rabbit	Cat# E18680	1:200	Spring Bioscience Corp., Pleasanton, CA 94566, USA.
Granzime B	Polyclonal, rabbit	Cat# E 2580	1:1,500	Spring Bioscience Corp., Pleasanton, CA 94566, USA.
Iba1	Polyclonal, rabbit	Cat# 019-19741	1:1,000	Wako, Richmond, VA 23285, USA.
Myeloperoxidase	Polyclonal, rabbit	Cat# IR511	Ready-to-use	Dako, Glostrup 2600, Denmark.
PAX5	Monoclonal	24/Pax-5	1:1,000	BD Biosciences, Lexington, KY 40502, USA.

DISCUSSION

The gross, histopathological, and immunohistochemical findings supported the diagnosis of lymphoma, which is an extranodal form of peripheral T-cell lymphoma according to the classification by the World Health Organization (Valli *et al.*, 2011). Lymphoma is a fairly common neoplasia in domestic dogs (Vail *et al.*, 2013), and a conclusive diagnosis requires immunophenotyping of neoplastic cells (Vail *et al.*, 2013). Several monoclonal antibodies have been demonstrated to cross-react with canine cell surface molecules, so they are suitable for phenotypic analysis of neoplastic cells in these cases (Lisowska *et al.*, 2018). In this report, we demonstrated that some of these markers also cross-react with cell surface molecules of the bush dog.

Immunohistochemistry has been extensively used for classifying canine lymphomas as well as for the differential diagnosis with other non-lymphoma round cell tumors. At least two markers are required for differentiation between B and T cell lymphoma (Burkhard and Bienzle, 2013). Usually, CD3 and CD79 or CD20 allows classification of approximately 80% of the cases (Guija de Arespacochaga *et al.*, 2007). Phenotyping of T cell lymphomas are often

based on expression of CD3, CD4, and CD8, whereas B cell lymphoma markers include CD20, CD21, CD79, and Pax5 (Caniatti *et al.*, 1996). Considering the immunophenotypic features in the present case, we favored the diagnostic hypothesis of a cytotoxic T cell lymphoma, since cells were CD3⁺ and expressed granzime B. Phenotyping of neoplastic cells in the present case was quite challenging since only scattered neoplastic cells were CD3 positive. CD3 staining in this case was mostly restricted to smaller lymphocytes, whereas most pleomorphic cells were negative. Neoplastic cells were diffusely negative for CD79a, CD20, and Pax5, clearly excluding a B cell phenotype.

Considering that a large number of neoplastic cells did not express CD3, while they were granzime B positive, a NK-cell lymphoma should be considered in the differential diagnosis. CD11d positivity with an extranodal distribution affecting the liver and the spleen allow us to include a hepatosplenic T-cell lymphoma in the differentials (Keller *et al.*, 2013). The T-cell population includes CD4⁺ T helper cells and CD8⁺ T cytotoxic cells. In addition to granzime B, T-cell intracytoplasmic antigen (TIA1) and perforin are suitable markers to identify cytotoxic T cells as well as NK cells, which express this protein in cytoplasmic

granules (Kanavaros *et al.*, 2000). Either rare or underdiagnosed, NK cells have been occasionally reported in dogs (Lane *et al.*, 2012).

To further characterize this kind of neoplasm, an expanded panel including CD5, CD7, CD8, CD16 and CD56 has proven useful in human patients (Miyata-Takata *et al.*, 2018). However, these antibodies do not react with formalin fixed, paraffin embedded tissue samples, and may not cross react with canine tissues. Human patients rarely develop NK/T lymphoma, which is a non-Hodgkin lymphoma, that affects mainly the upper respiratory and digestive tracts, and it is related to EB virus infection (Zhang *et al.*, 2018). There are no reports of this kind of lymphoma in domestic dogs, or in wild animals. Some Iba1 positive cells were scattered in the neoplastic tissue, indicating infiltration of histiocytic cells, which were not considered to be neoplastic.

Most of the canine lymphomas are B cell tumors, but T cell lymphomas usually have worst prognosis with average survival of 159 days, whereas B cell lymphomas have a survival period of 300 to 400 days after diagnosis and treatment (Zandvliet, 2016).

This is the first reported case of lymphoma in a bush dog (*Speothos venaticus*). In domestic dogs, lymphoma may affect any breed, although genetic or breed predisposition have been demonstrated (Pastor *et al.*, 2009). Even though lymphomas may occur at any age in domestic dogs, middle-aged and old dogs have a higher incidence, but there is no sex predisposition (Zandvliet, 2016). The bush dog in this case was housed at the zoological garden of Belo Horizonte (Fundação de Parques Municipais e Zoobotânica de Belo Horizonte), which is located within a metropolitan area. Although there are no specific causes for canine lymphomas, exposure to pollution may increase the risk of developing this type of neoplasm (Pastor *et al.*, 2009).

In conclusion, this is first reported case of lymphoma in a bush dog (*Speothos venaticus*), which is a South American endangered species. Our findings favored a diagnosis of cytotoxic T-cell lymphoma. In addition, this report demonstrated the suitability of several cell surface markers for differential diagnosis of round cell tumors in this species.

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