Case Report

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A Case of Visceral T Cell Lymphoma with Prominent Histiocyte Infiltration in a Dog

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Abstract A 13-year-old intact male English Springer Spaniel presented with anorexia. Physical examination revealed a palpable abdominal mass without peripheral lymphadenopathy. Ultrasonography revealed hepatosplenomegaly and a markedly enlarged hepatic lymph node. Fine-needle aspiration of the splenic and nodal lesions revealed atypical round cells admixed with numerous histiocytes. The dog was euthanized owing to deteriorating condition despite a month of chemotherapy with lomustine. Histopathology revealed obliteration of the normal architecture of the liver, spleen, kidney, and hepatic and mesenteric lymph nodes by $CD3^+$ neoplastic lymphocytes, accompanied by extensive $F4/80^+$ histiocytic infiltration. This report describes a rare presentation of T-cell lymphoma with prominent histiocytic infiltration that may initially be misdiagnosed as histiocytic neoplasia in a dog.

Key words case report, fine needle aspiration, histiocyte, T cell lymphoma, visceral involvement.

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Introduction

Lymphoma is common in dogs, making up 83% of all hematopoietic diseases (8). The most common form of canine lymphoma is the multicentric type; however, lymphoma can arise in virtually any location (7). Specific lymphoma entities, including hepatosplenic T-cell lymphoma, have been reported in dogs (2,4,5,9,16). Many cases of canine lymphoma are diagnosed using fine-needle aspiration (FNA) cytology, based on the predominant population of medium to large lymphocytes (14). In dogs, it is rare to observe nonlymphoid cells that can mask the presence of neoplastic lymphocytes on aspirated slides. An increasing number of cases have mentioned the prominent presence of histocytes in the histological examination of canine lymphomas (4,5,9,12,15,17). This study describes a dog with T-cell lymphoma and prominent histiocytic infiltration, as demonstrated by cytological and immunohistochemical examinations. Our case highlights the need for clinical awareness of histiocytic infiltration, which can lead to the misconception of atypical canine lymphoma as histiocytic neoplasia from splenic and nodal aspirates, especially in dogs with no peripheral lymphadenopathy.

Case Report

A 13-year-old intact male English Springer Spaniel was referred to Seoul National University Veterinary Medical Teaching Hospital to evaluate an abdominal mass. The patient had a history of anorexia, lethargy, vomiting, diarrhea, and weight loss. Physical examination revealed a palpable mass in the left upper abdomen. The body condition score (BCS) was five out of ten. Superficial lymph nodes were not enlarged. Complete blood count (CBC) results were within normal limits, with a hemoglobin of 14.7 g/dL (Reference interval [RI], 12.9-18.4 g/dL), hematocrit of 47.0% (RI, 37.1-57.0%), and RBC of 651 $\times$ $10^6/\mu L$ (RI, 570-880 $\times$ $10^6/\mu L$). Serum biochemical analysis revealed increased alkaline phosphatase activity (ALP, 603 U/L; RI, 8-100 U/L). Urinalysis results were within normal limits. Abdominal radiography revealed splenomegaly. Abdominal ultrasonography revealed hepatosplenomegaly with a hyperechoic hepatic nodule and multiple hypoechoic splenic nodules. Increased echogenicity was detected in the hepatic, splenic, and renal parenchymas. The hepatic and mesenteric lymph nodes were enlarged. FNA was performed on the splenic nodules and the hepatic lymph node. After Diff-Quik staining, splenic aspirates showed mild-to-moderate cellularity of individualized lymphoid round cells two to five times the erythrocyte diameter (Fig. 1A, B). The cells had a scant amount of moderately basophilic cytoplasm, with an occasional perinuclear pale zone. The nuclei were irregularly round to oval and frequently cleaved or convoluted with fine to coarsely stippled chromatin. Multiple prominent, bizarre nucleoli were observed in the nucleus (Fig. 1C). Up to three mitotic figures, including abnormal mitosis, were observed

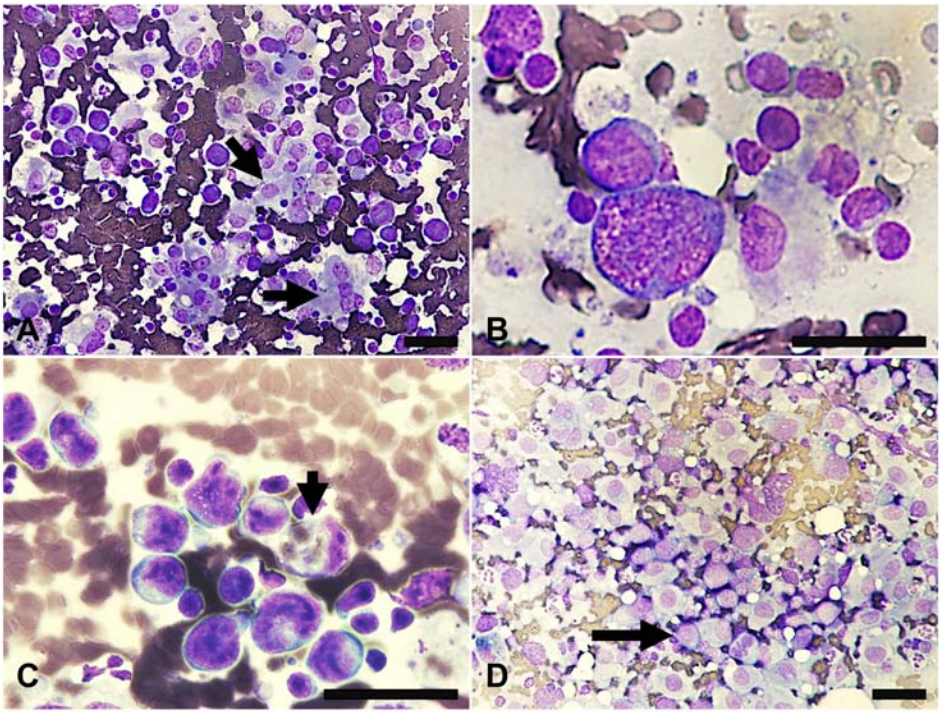

Fig. 1. Fine needle aspirates of splenic nodules and an enlarged hepatic lymph node in a dog. (A) Aspirates of splenic nodules reveal lymphoid cells presented with histiocytes having morphologic features, such as abundant pale cytoplasm and eccentrically located nucleus. Histiocytes show mild cellular atypia but display no epithelioid characteristics. The arrow indicates histiocyte- or mesenchymal cell-like morphology. In some locations on the same slide, large bizarre lymphoid cells (B) and erythrocyte cannibalism by the neoplastic cells (C) are present. The arrow indicates erythrophagocytosis. (D) Aspirates of hepatic lymph nodes reveal numerous histiocytes that obscure the presence of large lymphoid cells. The arrow indicates a large lymphocyte mixed with histiocytes. H&E stain, ×400 (A, D), ×1,000 (B, C). Scale bars = 40 μm (A, D), 50 μm (B, C).

per five fields at ×40 magnification. Erythrocytes were occasionally observed among these cells (Fig. 1C). Mixed with round lymphoid cells, moderate to high cellularity of individualized or aggregated cells showing histiocyte- or mesenchymal cell-like morphology was also observed (Fig. 1A, arrow). Histiocytes showed mild cellular atypia but displayed no epithelioid characteristics, such as abundant basophilic cytoplasm and a large polygonal shape. Depending on their location on the same slide, histiocyte-like cells often outnumber round lymphoid cells. The second population was oval to fusiform or stellate, with indistinct cell borders and eccentric nuclei. Vacuolation and erythrophagocytosis are rarely observed in histiocyte-like cells. The cells exhibited moderate anisocytosis with mild nuclear atypia, including coarse chromatin and prominent nucleoli. The aspirates of the hepatic lymph node also consisted of a heterogeneous population of medium-to-large neoplastic round lymphoid cells with predominant infiltration of histiocytes. Round neoplastic lymphoid cells were often difficult to detect because of the extensive number of admixed histiocyte-like cells (Fig. 1D). The cytological diagnosis was metastatic round cell tumor, with a primary differential diagnosis of lymphoid and histiocytic malignancies. Granulomatous lymphadenitis with reactive lymphoid hyperplasia was included in the differential diagnosis. The owner denied more definitive diagnostic approaches, such as ultrasound-guided core biopsy and immunocytochemistry. After a week, the dog was administered chemotherapy with lomustine (50 mg/m^2, PO) because it is known to be effective against malignant round cell tumors, including resistant lymphoma and histiocytic sarcoma. One week later, the dog did not show lethargy, vomiting, and diarrhea, but CBC showed mild leukopenia (3,850 cells/μL; RI, 5,200-17,000 cells/μL) with a hemoglobin of 13.3 g/dL (RI, 12.9-18.4 g/dL), hematocrit of 38.0% (RI, 37.1-57.0%), and RBC of 570 × 10^6/μL (RI, 570-880 × 10^6/μL). Two weeks later, the dog was brought to hospital with recurrent lethargy and anorexia. Abnormal serum biochemical parameters were alanine aminotransferase (198 U/L; RI, 6-90 U/L), aspartate aminotransferase (85 U/L; RI, 10-43 U/L), ALP (1,961 U/L; RI, 8-100 U/L), γ-glutamyl transpeptidase (172 U/L; RI, 0-14 U/L), total bilirubin (3.84 mg/dL; RI, 0-0.6 mg/dL), and total protein (4.5 g/dL; RI, 5-7.5 g/dL). Abdominal ultrasonographic examination revealed no biliary abnormalities. Differential diagnoses included intrahepatic cholestasis, cholangiohepatitis, and acute liver failure, based on the absence of evidence of hemolysis and extrahepatic cholestasis. Despite supportive medical care, including amoxicillin (22 mg/kg, PO, bid), metronidazole (7.5 mg/kg, IV, bid), tramadol (4 mg/kg, IV, bid), UDA (20 mg/kg, PO, bid), silymarin (20 mg/kg, PO, bid), lefotil (0.5 T/day, PO, bid), L-ornithine-L-aspartate (0.5 mL/kg, PO; bid) and fluid therapy, the overall condition of the dog continued to deteriorate by day 7, characterized by 15% weight reduction, progressive anemia (hematocrit of 25.6% with reticulocyte reproduction index of 0.96), and icterus. Euthanasia was decided based on poor prognosis. A necropsy was performed. Gross examination revealed multiple white to tan

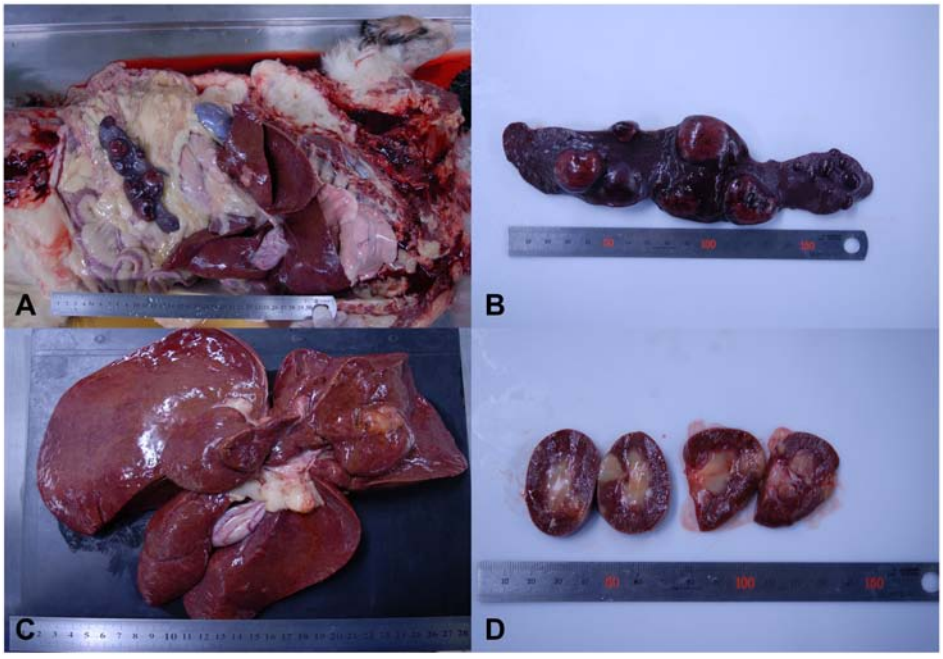

Fig. 2. Representative photographs showing the necropsy findings. (A) Opened thoracic and abdominal cavities. Multiple white to tan nodules were observed in various organs, including the (B) spleen, (C) liver, and (D) kidney.

nodules of varying sizes in the spleen, liver, kidneys, and intestines (Fig. 2). The mesenteric and hepatic lymph nodes were enlarged. On the cut surface, these nodular lesions were light tan to white and occasionally reddish with hemorrhage. The nodules were well-demarcated from the surrounding parenchyma. The liver was diffusely enlarged with a blunt margin. The oral mucosa and abdominal adipose tissues were slightly yellowish, and ascites were observed. Other parenchymal organs were unremarkable. Representative tissue samples were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin wax. Three micrometer sections were prepared and stained with hematoxylin and eosin (H&E). In most tissues, neoplastic cells exhibit vascular tropism. Microscopically, the nodules consisted of neoplastic proliferation of round cells (Fig. 3A), obliterating the normal architecture of the spleen, liver, kidney, small intestine, and mesenteric lymph nodes (Fig. 3B-F). The neoplastic cells were pleomorphic and contained a small amount of eosinophilic cytoplasm and a hyperchromatic round nucleus with a high degree of anisokaryosis. The nuclei contained a few prominent nucleoli. There were five to eight mitoses per ×400 high-power fields. Neoplastic cells were also found in blood and lymphatic vessels. Consistent with the cytological findings, the neoplastic cells were extensively mixed with histiocyte-like cells that did not show malignant cellular features. Neither multinucleated cells nor distinct granuloma formation were observed in the sections. However, marked vacuolar hepatopathy was observed. Based on the gross and histological findings, the neoplasm was presumptively diagnosed as a round cell tumor (probable lymphoma) with extensive histiocytic infiltration. Systemic dissemination of lymphoma to major organs is considered a rapidly deteriorating condition. Vacuolar hepatopathy and hepatic parenchymal effacement are considered causes of hepatic insufficiency, icterus, and ascites. To validate this diagnosis, immunohistochemistry (IHC) was performed on serial H&E-stained sections (Fig. 4A). After hydration and heat-induced antigen retrieval, endogenous peroxidase was depleted by incubating slides in 0.3% hydrogen peroxide for 1 hour. Sections were incubated at 4°C overnight with CD3 (A0452; Dako, CA, USA), CD79α (M7051; Dako), and F4/80 (sc-26643-R; Santa Cruz Biotechnology, TX, USA), and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. All sections were visualized using a 3,3′-diaminobenzidine solution and then counterstained with Mayer's hematoxylin. The round cell-morphology neoplastic cells were CD3-positive and CD79α-negative, and histiocyte-like cells were F4/80-positive (Fig. 4B-D). Based on the cytology,

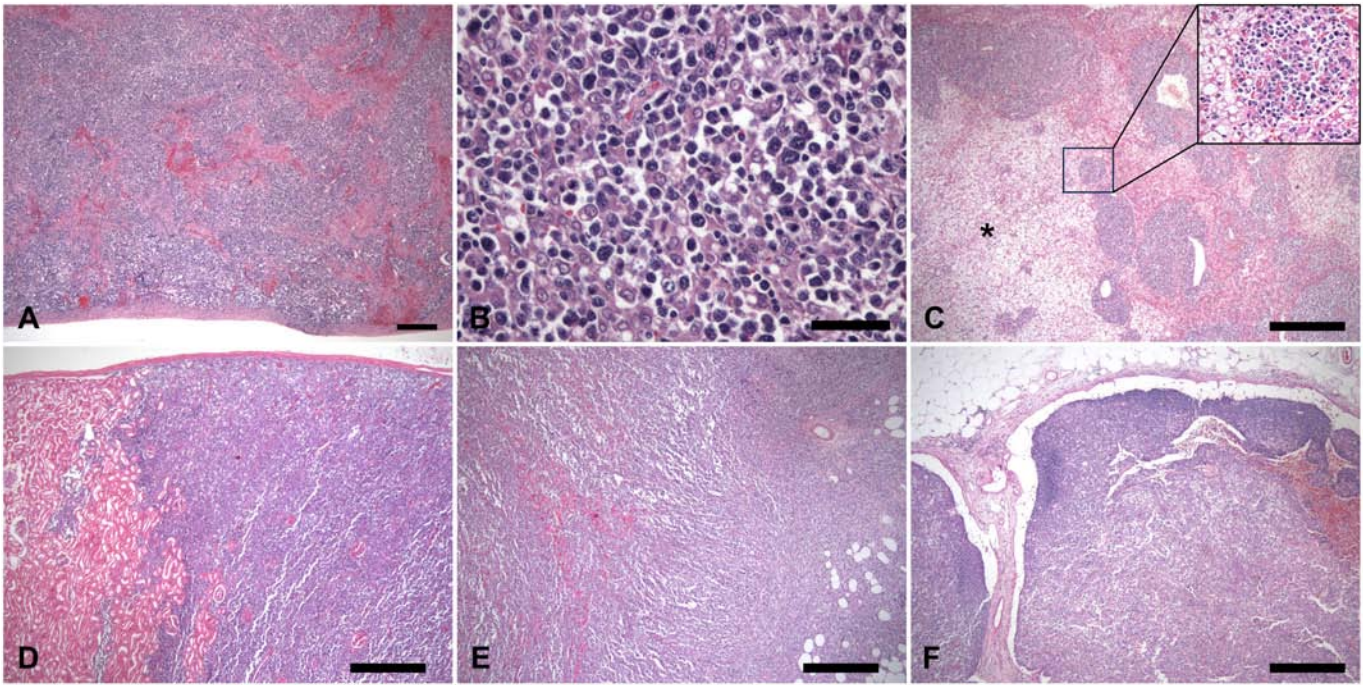

Fig. 3. Histological examination of the nodules of multiple organs in a dog. Neoplastic cells are extensively infiltrating and obliterate normal parenchymal structures of (A) spleen, (B) hepatic lymph node, (C) liver, (D) kidney, (E) small intestine, and (F) mesenteric lymph node. (B) Prominent histiocytes are mixed with neoplastic lymphocytes. (C) Live parenchyma shows marked vacuolar hepatopathy (asterisk and insert). H&E stain, A: ×40, Scale bars = 500 μm, B, C, D, E, F: Scale bars = 40 μm.

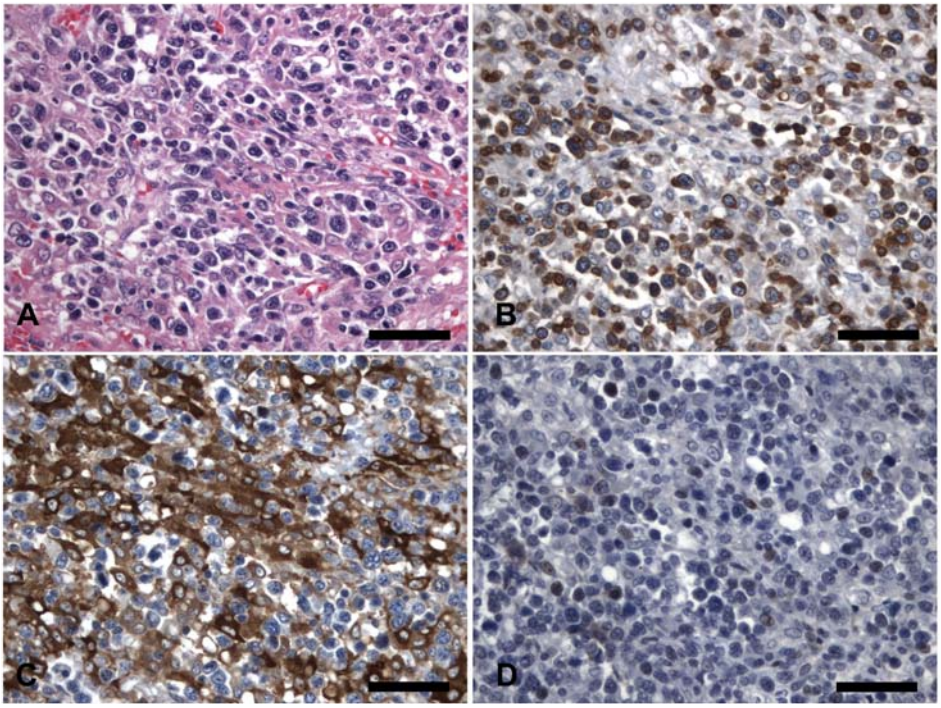

Fig. 4. Immunohistochemistry of lymphoma in a dog. (A) The same H&E-stained section for (B-D). (B) The neoplastic cells are specifically positive for CD3. (C) The histiocytes are positive for F4/80 but negative for CD3 and CD79α. (D) The neoplastic cells are negative for CD79α. H&E stain, A: ×400, Scale bars = 40 μm, IHC stain, B, C, D: ×400, Scale bars = 40 μm.

histopathology, and immunohistochemistry results, the dog was diagnosed with visceral T-cell lymphoma with prominent histiocytic infiltration.

Discussion

Emerging cases of canine lymphomas have documented the clinical importance of histiocytic infiltration; however, its clinical relevance has not been assessed (4,5,9,12,15,17). No previous cytological studies have shown that prominent histiocytic infiltration can mask the presence of neoplastic lymphocytes in the splenic nodules and lymph nodes of dogs. Histiocytic proliferation has great potential to spatially mask the presence of neoplastic lymphocytes, leading to an initial misdiagnosis of reactive histiocytosis or histiocytic neoplasia. Our case raises awareness of prominent histiocytic infiltration in canine T-cell lymphomas. Pleomorphic large T-cell lymphoma shares cytological features with histiocytic malignancies, including bizarre cell morphology and phagocytosis (2,4,5,9,10), consistent with our case. Practitioners strongly recommend avoiding areas with high cellularity of aggregated cells, such as histiocytes, in lymphoid tissues, especially the spleen, and navigating low-to-medium power fields, where typical large lymphocytes characterized by scant cytoplasm are observed. Molecular diagnostic techniques, including flow cytometry-based immunophenotyping and antigen rearrangement in T- and B-cell receptors, can help identify clonal expansion in lymphocytes. Caution is warranted when testing the polymerase chain reaction for antigen receptor rearrangements because myeloid lineage cells may produce false-positive and false-negative results due to their T and B cell receptor genes.

The clinical relevance of histiocytic infiltration is completely unknown in veterinary medicine; however, it is interpreted as a secondary reactive response to tumor growth, extramedullary hematopoiesis (EMH), erythrocytic phagocytosis (4,5,9), and importantly protumoral activity (17). In our case, EMH was absent in the spleen and liver, and histiocytic phagocytosis was unremarkable. Most canine lymphomas that are accompanied by histiocytic infiltration are of T-cell origin, which is consistent with our case. F4/80 is a reliable macrophage marker in humans, mice, and dogs (1). T-cell lymphoma, which recruits tumor-infiltrating histiocytes, shows a poor prognosis due to immune suppression in humans (11). Thus, we postulated that infiltrating histiocytes promote the aggressiveness of T-cell lymphoma in dogs. Future molecular studies should address whether histiocytes support the aggressive clinical course and poor outcome of canine visceral T-lymphoma via indirect or direct tumor-histiocyte interactions, as suggested in human medicine (3).

Our case shares numerous clinical, clinicopathological, and histological features with hepatosplenic T-cell lymphoma in dogs, including anatomic location, aggressive progression, and vascular tropism (2,4,5,16). Immunohistochemical stain-

ing of γδ T-cell receptors may indicate this distinct type of T-cell lymphoma. Frequent extranodal involvement of the liver and spleen and the absence of peripheral lymphadenopathy were unusual clinical features in our case of T-cell lymphoma. Marked vacuolar hepatopathy, another unusual aspect, appears to be secondary to extensive infiltration of T-cell lymphoma that compresses and distorts the normal hepatic parenchyma. Canine lymphoma can be accompanied by marked vacuolar hepatopathy; however, it is uncommon (13). Considering the relatively short duration of lomustine treatment (6), it was unlikely to be responsible for the magnitude of vacuolar hepatopathy observed in this study. Neoplastic erythrophagocytosis and anemia of inflammatory diseases may simultaneously contribute to anemia with borderline regeneration (5).

Conclusions

Herein, we report the clinical, clinicopathological, histological, and immunohistochemical findings of T-cell lymphoma in a dog without superficial lymphadenopathy. We highlight the need for increased clinical awareness of histiocytic infiltration in canine splenic and nodal lymphomas. We believe that the results presented here will help veterinary practitioners recognize cases of atypical lymphoma in their diagnostic routines. Finally, we suggest that extensive molecular studies address the clinical importance of histiocytic infiltration in canine T-cell lymphoma progression.

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Conflicts of Interest

The authors have no conflicting interests.

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