CASE REPORT

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Pericardial effusion in a dog due to T-cell lymphoma of granular lymphocyte type

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Abstract
Pericardial effusions in dogs are most often diagnosed as haemorrhagic and idiopathic. Pericardial effusions secondary to an underlying neoplastic process are infrequently diagnosed, as neoplastic cells are rarely observed in a sample of the effusion. In the present report, we describe a 9-year-old dog with pericardial effusion due to T-cell lymphoma of granular lymphocyte type. Immunophenotyping and molecular clonality PCR were performed to confirm the cytologic diagnosis. To our knowledge, this is the first report of pericardial effusion in a dog due to T-cell lymphoma of granular lymphocyte type.

KEYWORDS
dog, immunophenotyping, lymphoma of granular lymphocyte type, molecular clonality PCR, pericardial effusion, T-cell lymphoma

1 | INTRODUCTION

Pericardial effusions in dogs are most often diagnosed as haemorrhagic and idiopathic (90%), with fewer cases diagnosed as neoplastic (4.6%), infectious (3.1%), or other (2.3%) based on microscopic evaluation according to a recent study (Cagle et al., 2014). The three most common neoplastic causes of pericardial effusion in dogs are myocardial hemangiosarcoma, chemodectoma, and mesothelioma (MacGregor et al., 2005), although the cells of the first two tumour types are typically not present on microscopic evaluation of the effusions, and a definitive diagnosis of mesothelioma ante-mortem is problematic. Pericardial effusion due to lymphoma is rare in dogs, occurring in only 1.9% of pericardial fluid analyses in one study (Cagle et al., 2014) and 1.2% of pericardial fluid analyses when data from three separate studies were examined (Berg et al., 1984; Dunning et al., 1998; Sisson et al., 1984). Another retrospective study found that only 0.17% of dogs presenting with cardiothoracic disease across three tertiary veterinary teaching hospitals had primary cardiac lymphoma (MacGregor et al., 2005). Four of the 12 affected dogs in that study had T-cell lymphoma, and one had

B-cell lymphoma, determined via immunohistochemical and immunocytochemical immunophenotyping (MacGregor et al., 2005). However, the T-cell lymphomas present in these pericardial effusions were not described as granular, and to the authors' knowledge, this is the first report of a pericardial effusion in a dog due to T-cell lymphoma of granular lymphocyte type.

2 | CASE PRESENTATION

A 9-year-old 25-kg (55 lb) spayed female Labrador Retriever-Poodle crossbreed dog was examined by the emergency service at a veterinary teaching hospital for diarrhoea, vomiting, inappetence, and an enlarged abdomen. Five months earlier, the dog was diagnosed with pituitary-dependent hyperadrenocorticism via a low-dose dexamethasone suppression test at the same veterinary teaching hospital. At that time, the physical examination was overall unremarkable, with the exception of bilateral lenticular sclerosis and a mildly pendulous abdomen. A complete blood count (CBC) and serum biochemistry

panel were unremarkable, and atypical cells were not observed on blood smear examination. An abdominal ultrasound was performed and revealed mildly enlarged kidneys bilaterally, an enlarged liver with multifocal hyperechoic nodules and an enlarged spleen. Fine needle aspiration of these lesions was not performed at that time.

On physical examination, the dog was bright, alert, and responsive and had pale mucus membranes. Mild cranial organomegaly and an abdominal fluid wave were detected on palpation. Point-of-care ultrasound was performed on the abdominal and thoracic cavities. There was a moderate amount of fluid in the abdomen, and therapeutic abdominocentesis was performed without cytologic evaluation of the fluid. Pericardial effusion was also present, and during pericardiocentesis, the patient developed severe atrial flutter. The patient was administered 2% lidocaine IV during the procedure, and oral sotalol was prescribed to prevent future atrial flutter events. Echocardiography revealed pericardial effusion with cardiac tamponade and a 2×1 cm hyperechoic structure near the pulmonary artery, which was interpreted as either a clot or a mass. This mass was not aspirated due to patient instability. Approximately 600 ml of serosanguineous fluid was removed from the pericardial space and submitted to the clinical pathology laboratory for analysis. No further diagnostics were pursued, and the patient was discharged with an appointment for recheck in 1 week.

The pericardial fluid had a protein concentration of 4.3 g/dl (RI < 2.5 g/dl), a total nucleated cell count of 23,500 cells/μl (RI < 1,500 cells/μl), and 1.3 million red blood cells (RBCs)/μl (RI 0 cells/μl). A Wright–Giemsa stained direct smear of the fluid had a pale blue background with increased nucleated cellularity (Figure 1a). Nucleated cells were predominantly large round cells that had round to ameboid nuclei approximately 2–3 RBCs in diameter, finely clumped chromatin, multiple variably prominent nucleoli, and a moderate volume of mid to deep blue cytoplasm that often contained numerous small punctate vacuoles and sometimes contained a perinuclear packet of fine pink granules (Figure 1b). There were also numerous cytophagic macrophages, along with few small, well-differentiated lymphocytes, and non-degenerate neutrophils. Moderate numbers of reactive mesothelial cells were also observed. Cytologic interpretation of the fluid was "possible lymphoma with haemorrhage," and immunophenotyping and molecular clonality PCR (PARR) were recommended to further characterize the cells in the fluid.

The dog was seen 1 week later for the scheduled recheck appointment and had developed increased respiratory effort and decreased lung sounds ventrally. Ultrasonography detected pleural effusion that was not present at the visit a week earlier, and thoracocentesis was performed. Approximately 300 ml of serosanguineous fluid was removed from the right side of the thorax and submitted for fluid analysis. The pleural fluid sample had a protein concentration of 5.8 g/dl (RI < 2.5 g/dl), a total nucleated cell count of 23,420 cells/μl (RI < 1,500 cells/μl), and 140,000 RBCs/μl (RI 0 cells/μl). On microscopic examination, the predominant cell type consisted of large round cells that were morphologically similar to those present in the pericardial fluid sample one week prior (Figure 2). The dog was diagnosed
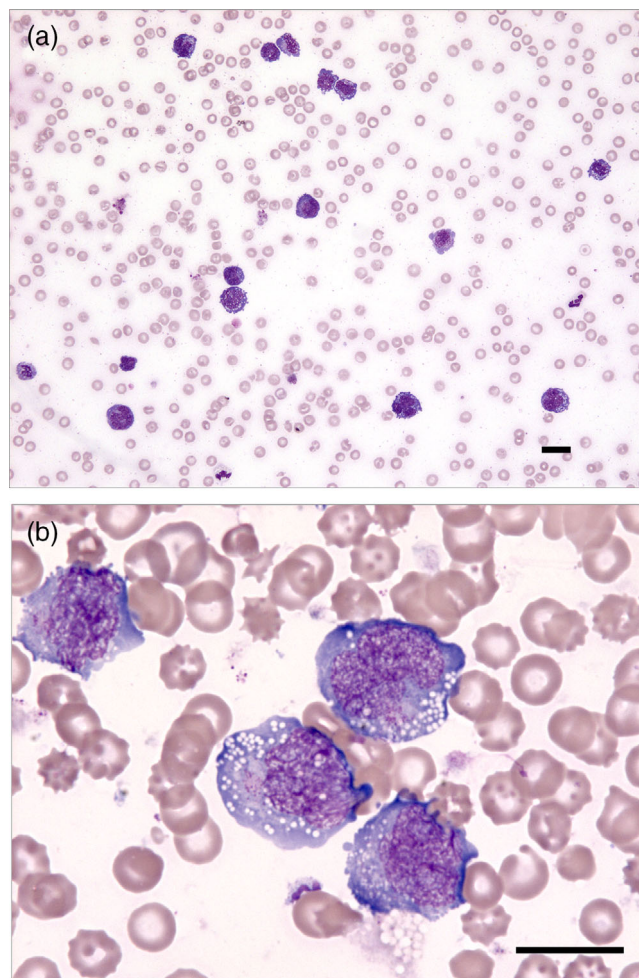
FIGURE 1 (a) Photomicrograph of a pericardial fluid sample collected from a 9-year-old Labrador Retriever-Poodle cross breed dog that presented for vomiting, diarrhoea, and a distended abdomen. There are numerous large round cells that have moderate volumes of mid to dark blue cytoplasm that often contain numerous punctate vacuoles. Wright-Giemsa stain; bar = 20 μM. (b) Photomicrograph of a sediment preparation of the pericardial fluid sample. Some of the large round cells also have fine, bright pink cytoplasmic granules that packet in a perinuclear location. Wright-Giemsa stain; bar = 20 μM

with neoplastic pericardial and pleural effusions due to lymphoma of granular lymphocyte type.

To further characterize the neoplastic cells, immunophenotyping using anti-CD3 (T lymphocyte marker), anti-CD79a (B lymphocyte marker), anti-CD11d (marker of granular lymphocytes and macrophages in hematopoietic sites—splenic red pulp, bone marrow, lymph node medullary sinuses), anti-CD11c (myeloid lineage marker, including dendritic cells), and anti-CD11b (myeloid lineage marker) antibodies was performed on smears of the pericardial fluid. The neoplastic cells were weakly to moderately CD3 positive cytoplasmically and did not express CD79a, CD11b, CD11c, or CD11d. They were also subsequently negative for CD34 expression and alkaline phosphatase activity, the latter being shown to be weakly positive in T-ALL
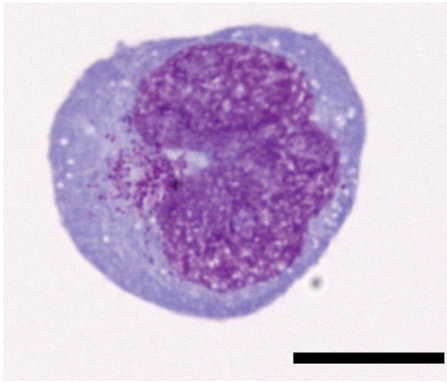
FIGURE 2 Photomicrograph of the thoracic effusion that developed 1 week after original presentation in a 9-year-old Labrador Retriever-Poodle cross breed dog that presented with pericardial effusion previously. The large round cells present in the thoracic fluid are morphologically identical to those observed in the pericardial fluid. They have fine, bright pink cytoplasmic granules that packet in a perinuclear location. Wright-Giemsa stain; bar = 10 μM

(Vernau & Moore, 1999). Molecular clonality PCR (PARR) was also performed in duplicate on the pericardial fluid to determine if the neoplastic cells had a clonal T-cell receptor gene rearrangement (Figure 3) (Keller & Moore, 2012). The neoplastic cells had a marked and reproducible T-cell receptor gamma clonal rearrangement. T-cell molecular clonality PCR was also performed in duplicate on the pleural fluid, and a reproducible clonal rearrangement was also present, identical to that in the pericardial fluid (Figure 3). No further diagnostic workup was pursued, and the patient was lost to follow-up.

3 | DISCUSSION

Large granular lymphocyte (LGL) neoplasia is of T-cell or natural killer (NK) cell origin and may manifest as lymphoma or leukaemia. Various types of T-cell lymphoma of granular lymphocyte type have been reported in dogs, including those that arise in the splenic red pulp (hepatosplenic lymphoma [HS-TCL], CD11d+), liver (hepatocytotrophic lymphoma [HC-TCL], CD11d-), and gastrointestinal (GI) tract (enteropathy-associated T-cell lymphoma type 1 [EATCL type 1], CD11d-) (Keller et al., 2013; Matsumoto et al., 2019). EATCL may have some degree of blood involvement, but HC-TCL and HS-TCL typically do not (Keller et al., 2013). Most granular lymphocyte leukaemias in
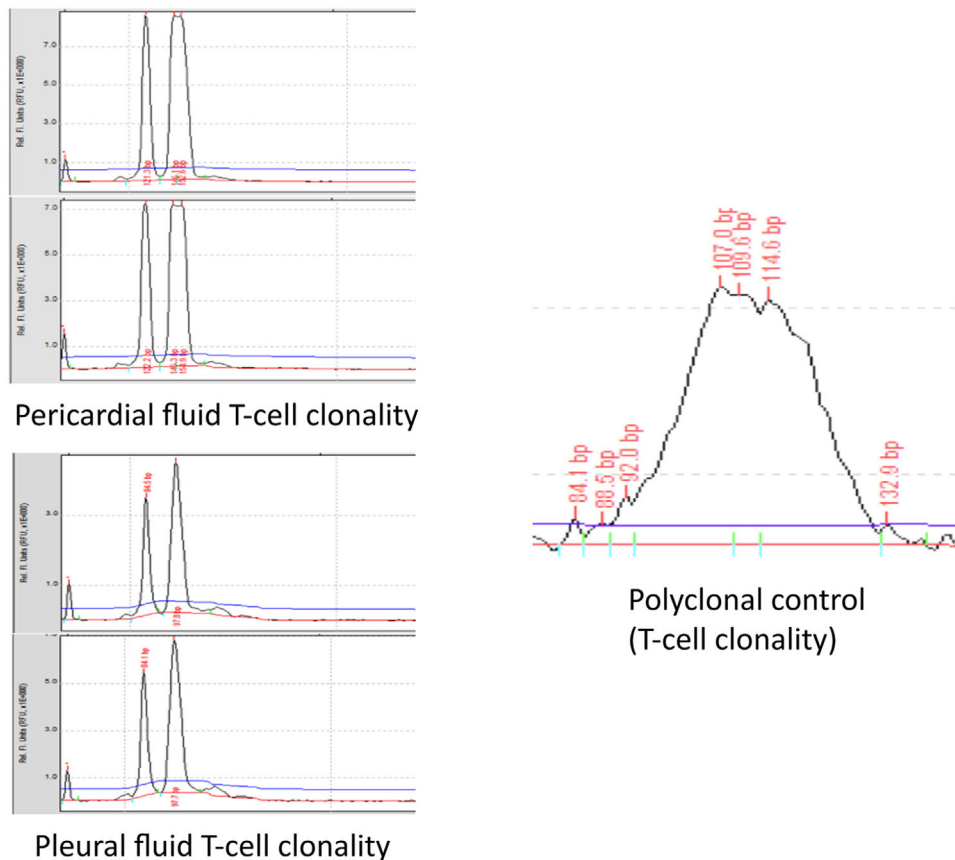
(T-cell acute lymphoblastic leukaemia) in one study (Stokol et al., 2015) and the former being strongly and consistently expressed in acute myeloid leukaemias and B-cell acute lymphoblastic leukaemia (B-ALL)

Pericardial fluid T-cell clonality

Pleural fluid T-cell clonality

Polyclonal control
(T-cell clonality)

FIGURE 3 T-cell molecular clonality PCR electropherogram traces (run in duplicate) from both the pericardial and pleural effusions present in a 9-year-old Labrador Retriever-Poodle cross breed dog. Reproducible identical clonal electropherograms are indicative of the same T-cell lymphoma (identical clones) in both sites

dogs are of cytotoxic T-cell origin (CD3+CD8+) and arise from the splenic red pulp. As such, they are also CD11d+ (Vernau & Moore, 1999). There are no reports of primary cardiac or pericardial lymphoma of granular lymphocyte type in animals, but a human case of NK-cell LGL lymphoma in a pericardial effusion secondary to Epstein–Barr virus (EBV) infection has been reported (Hisatake et al., 1996). An NK-cell origin was excluded in this instance, as the neoplastic lymphocytes had a clonal T-cell receptor gene rearrangement. Natural killer cells do not rearrange their T-cell antigen receptor genes (Caligiuri, 2008). Increased proportions of cardiac CD8+ T cells have been reported with aging in mice (Ramos et al., 2017), and whether this increase occurs in aging dogs, or if those cells could undergo malignant transformation, remains to be determined.

In the dog described here, there were no GI abnormalities or abdominal lymphadenomegaly present on ultrasound. This makes primary EATCL an unlikely origin for the neoplastic LGLs in this case. Moreover, cardiac involvement is uncommon with either HC-TCL or HS-TCL (Keller et al., 2013), and HS-TCL in dogs is usually CD11d+. Consequently, both HS-TCL and HC-TCL are considered unlikely primary sources as well. Although a CBC was not performed in this dog when it presented with pericardial effusion, a primary acute leukaemia of granular lymphocyte type is also considered unlikely due to the immunophenotype (CD11d-, CD34-) and lack of apparent more widespread involvement. As such, primary cardiac or pericardial T-cell lymphoma of granular lymphocyte type was considered most likely. However, without a CBC and full staging at the time the dog developed the neoplastic (LGL) effusion, or a subsequent post-mortem, this cannot be confirmed, and lymphoma of granular lymphocyte type arising elsewhere, with secondary cardiac or pericardial involvement, remains a possibility.

ACKNOWLEDGEMENTS

ETHICS STATEMENT

The dog described in this report was a client-owned patient presented for care at the UC Davis VMTH. Informed owner consent was received for any possible research use of all diagnostic samples acquired from the patient during hospitalization.

AUTHOR CONTRIBUTIONS

Conceptualization, data curation, formal analysis, investigation, methodology, visualization, and writing—original draft: Demitria Vasilatis. *Data curation, formal analysis, investigation, methodology, project administration, writing—review and editing*: William Vernau.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

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