Short communication

SPONTANEOUSLY ARISING DISEASE

Short Title: Peliosis Hepatis with Chylous Ascites in a Dog

Peliosis Hepatis with Chylous Ascites in a Dog

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Summary

A 6-year-old spayed female Toy Poodle dog was referred to the Hokkaido University Veterinary Teaching Hospital for abdominal distension. Abdominocentesis yielded ascitic fluid that had a mildly increased total protein concentration and a 2.7-fold higher triglyceride concentration than plasma, and was interpreted as chylous ascites. The patient had enlarged liver which contained multiple, small, nodular masses and cyst-like structures. Microscopically, these lesions were multifocal dilated spaces containing lymphocytes, endothelial cells, fibrin and islands of hepatocytes. Increased αSMA-positive cells were observed in hepatic sinusoids. Based on these findings, we diagnosed peliosis hepatis with chylous ascites, which is likely to have been due to

lymphangiectasia and disrupted hepatic sinusoids. Neither *Bartonella* sp DNA nor mutations in *ACVRL1* and *MTM1* genes were detected although there was a 47-fold increase in hepatic *ACVRL1* expression compared to age-matched control liver. To the authors' knowledge, this is the first report of chylous ascites resulting from peliosis hepatis in any species.

Keywords: ACVRL1; chylous ascites; dog; peliosis hepatis

Peliosis hepatis (PH) is a rare vascular disease characterized by numerous, randomly dispersed, blood-filled cavities in the liver. The diameter of the cavities typically varies from a few mm to 3 cm. PH has been reported in dogs, cats, rats, cattle and humans (Degott *et al*, 1978; Zafrani *et al*, 1984; Maxie, 2007), and may occur at any age but is more common in middle-aged or old humans and dogs. Male predominance was documented in humans, but PH has been suggested to be more common in female dogs (Degott *et al*, 1978; Inoue *et al*, 1988; Izumi *et al*, 1994).

In humans, PH has been associated with activin receptor-like kinase 1 (*ACVRL1*), myotubularin 1 (*MTM1*) and endoglin (*ENG*) mutations or repression (Laporte *et al*, 2000; Kjeldsen *et al*, 2005). In dogs, *Bartonella henselae*, the causative agent of cat-scratch disease, was reported to be a possible cause of PH (Kitchell *et al*, 2000). Interestingly, in cats, *B. henselae* infection is not associated with the disease (Buchmann *et al*, 2010) and no association has been made in other species. Diagnosis of PH in humans is challenging (Daniele Crocetti *et al*, 2015; Dai *et al*, 2017) and computed tomography (CT) and magnetic resonance imaging (MRI) do not give conclusive results (Perkocha *et al*, 1990). In addition, PH grossly resembles, and can be misdiagnosed as, a metastatic tumour or liver abscess (Jensen *et al*, 2000).

Chylous ascites is characterized by the accumulation of triglyceride-rich peritoneal fluid with a milky appearance (Al-Busafi *et al*, 2014). In humans, chylous ascites has been documented in congenital diseases such as lymphatic hypoplasia, intestinal lymphangiectasia,

neoplastic diseases such as lymphoma and Kaposi sarcoma, and in infectious diseases such as tuberculosis and filariasis (Bhardwaj *et al*, 2018). Chylous ascites is rare in animals and has been reported in dogs and cats with malignant neoplastic disease, lymphangiectasia, lymphatic obstruction and lymphatic rupture (Fossum *et al*, 1992; Lott *et al*, 2015; Hatch *et al*, 2018). PH has not been reported as a cause of chylous ascites in any species.

A 6-year-old spayed female Toy Poodle dog was referred to the Hokkaido University Veterinary Teaching Hospital for abdominal distension (Supplementary Fig. 1A). Abdominocentesis yielded reddish, opaque abdominal fluid. Fluid analysis revealed nucleated cell counts of 1,300 cells/μL, composed mainly of small lymphocytes, along with traces of neutrophils and macrophages with a few red blood cells (8,000 cells/μL) (Supplementary Fig. 1B). The centrifuged fluid was whitish yellow with a slightly increased total protein concentration (2.6 g/dL). Triglyceride concentration in the abdominal fluid (149 mg/dL) was 2.7 times higher than in plasma (55 mg/dL). Based on these findings, chylous ascites was diagnosed. Ultrasonography revealed that the liver was larger than normal and contained multiple, small, nodular masses, and cyst-like structures (Supplementary Figs. 1C and D). The patient was treated continuously with spironolactone and prednisone to manage the ascites, but the clinical condition worsened and the dog died 3 weeks after the initial presentation.

At necropsy, irregularly circumscribed red foci of up to 3 cm × 2.5 cm in diameter were observed multifocally in all lobes of the liver (Fig. 1). The dark-red foci had a cyst-like appearance and blood-tinged fluid oozed from the cut surface. Microscopic examination revealed that these lesions were multifocal dilated spaces containing lymphocytes, endothelial cells, fibrin and islands of hepatocytes (Fig. 2). Some dilated spaces also contained copious amounts of red blood cells and blood clots. The lymph vessels in portal regions were moderately dilated compared with a normal control liver from an age-matched, female, French Bulldog (Supplementary Figs. 2A and 2B). The number of αSMA-positive cells was higher in the hepatic sinusoids of the patient's liver compared to the control liver (Fig. 3). Jones'

79 methenamine silver staining of liver sections revealed disruption of reticulin fibres and
80 thickening of the basement membrane in the perisinusoidal region compared to the normal age-
81 matched control (Fig. 4).
82 DNA was extracted from frozen liver and an age-matched control using the QIAamp
83 DNA Mini-kit (Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) was
84 performed for *Bartonella* species as described (Jensen *et al*, 2000; Johnson *et al*, 2003) and was
85 negative (Supplementary Fig. 3A). Total RNA was extracted with Nucleospin® RNA isolation
86 kit (Macherey-Nagel, Düren, Germany) and synthesis of cDNA was performed using the
87 PrimeScript™ Reverse Transcriptase (Takara Bio, Kusatsu, Japan) following the manufacturers'
88 instructions. Canine myotubularin 1 (*MTM1*) and activin receptor-like kinase 1 (*ACVRL1*)
89 coding sequences were cloned using the primers listed in Supplementary Table 1. Mutations in
90 the coding sequences of these genes were not detected by sequential analysis and alignment of
91 the cloned sequences with the canine reference genes on Ensemble database. Reverse
92 transcription quantitative PCR (RT-qPCR) assay was performed to determine the mRNA
93 expression of *ACVRL1* and *MTM1* genes. Results showed that the liver had a 47-fold increased
94 expression of *ACVRL1* compared with the age-matched control liver, while no significant
95 difference was observed in *MTM1* expression (Supplementary Fig. 3B).
96 The definitive diagnosis of PH was made after diagnostic imaging, histopathological
97 examination with special staining for reticulin, and immunohistochemistry for α-SMA. The
98 chylous ascites was likely due to the lymphangiectasia and disrupted hepatic sinusoids in the
99 liver. The space of Disse is the major route of lymph flow in the liver (Ohtani and Ohtani, 2008)
100 and, in this case, the sinusoid basement membrane was disrupted, which likely allowed
101 accumulated lymph to flow into the sinusoid resulting in the formation of cyst-like spaces
102 containing blood and lymph. We hypothesize that the accumulated lymph and blood entered the
103 abdominal cavity due to hepatic rupture, which led to the accumulation of chylous ascites. Other
104 possible causes of chylous ascites such as lymphatic hypoplasia and neoplastia were not

supported by physical examination, haematology, blood chemistry, diagnostic imaging, necropsy or histological analyses.

Although mutations in *MTM1* and *ACVRL1* coding sequences were not detected in this case, RT-qPCR assay revealed a significant increase in the expression of *ACVRL1* in liver compared with the age-matched control liver. ACVRL1, also known as ALK1, is a receptor in the TGF-β and BMP9 signalling pathways (Scharpfenecker *et al*, 2007). Considering most *ACVRL1* mutations in humans are dysfunctional, loss of function of TGF-β or BMP9 signalling is probably responsible for PH development. Since there was no mutation of the coding region in *ACVRL1* in the present case, these downstream signalling pathways of *ACVRL1* might be non-functional (Gu *et al*, 2006; Ricard *et al*, 2010; Ruiz *et al*, 2017). This can result in a compensatory increase in *ACVRL1* expression.

Another hypothesis is that the increased ACVRL1 is the direct cause of PH. ACVRL1 expression is induced during angiogenesis but is diminished when vessels mature, which suggests that it has a role in inhibiting angiogenesis and in stabilizing vessel structural integrity (Seki Tsugio *et al*, 2003; Tual-Chalot *et al*, 2014). However, one study reported that ACVRL1 could stimulate proliferation and migration of normal endothelial cells (Li *et al*, 2008). Overexpression of ACVRL1 could dysregulate angiogenesis, leading to disorganized hepatic sinusoids. The likely cause of the increased *ACVRL1* expression in this case are mutations in non-coding sequence regions, including the promoter or the introns which could have been missed by the sequential analysis. Another possible cause of increased *ACVRL1* expression is mutations in the genes coding for the transcription factors for the *ACVRL1* gene or for the *ACVRL1* upstream pathway genes. However, the role of ACVRL1 in canine PH is unclear.

To the authors' knowledge, this is the first report of chylous ascites resulting from PH in a dog. This study presents several diagnostic options which are essential for PH diagnosis. As the exact mechanism of PH is unknown, further studies on its pathogenesis and treatment options are warranted.

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Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

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Figure legends

Fig. 1. Peliosis hepatis, liver, dog. Numerous, irregularly lined, randomly dispersed, blood-filled cavities throughout cranial aspect of liver. Cyst rupture in caudate lobe (arrow).

Fig. 2. Peliosis hepatis, liver, dog. Cyst contains lymphocytes, islands of hepatocytes, endothelial cells and few erythrocytes. HE.

Fig. 3. Peliosis hepatis, liver, dog. Increased intensity of immunolabelling of αSMA in sinusoids (left) compared with age-matched control liver (right).

Fig. 4. Peliosis hepatis, liver, dog. Reticulin fibre disruption and thickening of basement membrane in perisinusoidal regions (left) compared with age-matched control liver (right). Jones' methenamine silver.

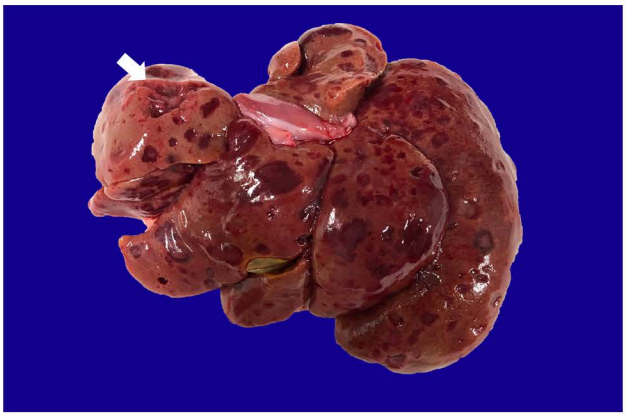

Fig. 1

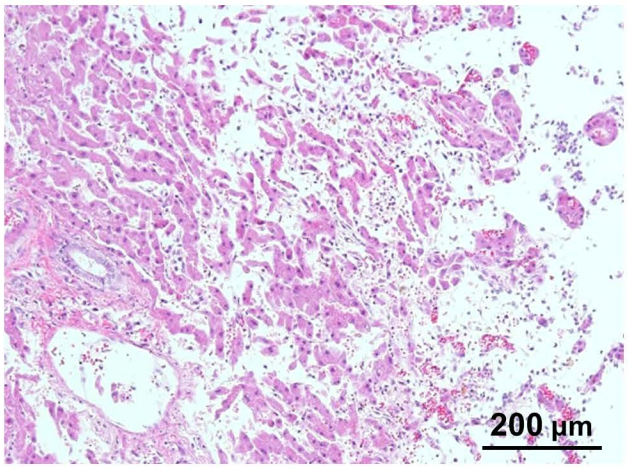

Fig. 2

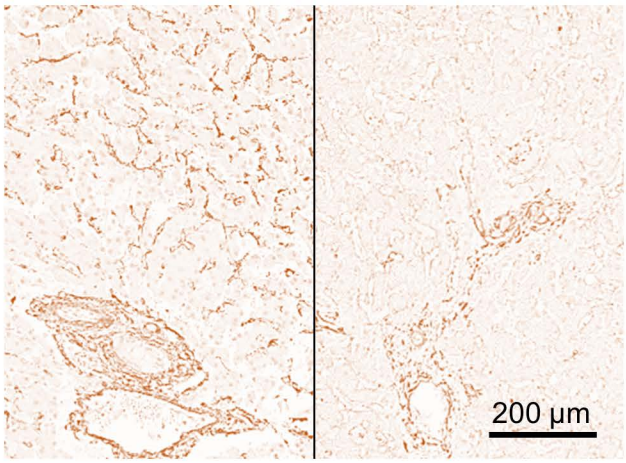

Fig. 3

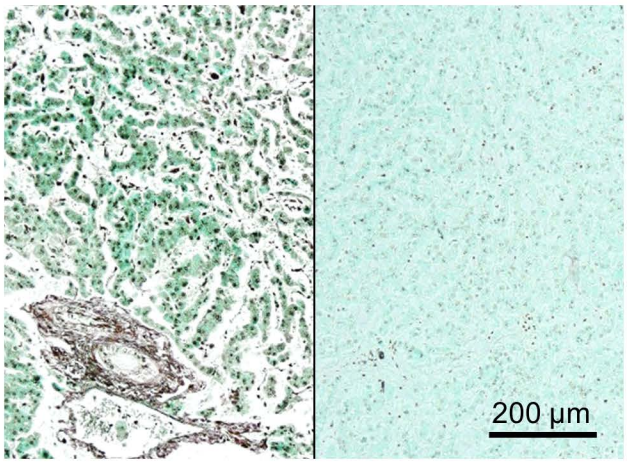

Fig. 4