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Lipoprotein profile of pleural and peritoneal transudates in dogs and cats

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Abstract

Background: Current diagnostic evaluation of transudative effusions rarely aids in identifying an underlying etiology. Lipoproteins in the fluid might reflect the site or nature of vessel involvement.

Objectives: Improve the classification and diagnostic utility of pleural and peritoneal transudates in dogs and cats by investigating lipoprotein patterns in effusions. Compare these patterns with other peritonaeal and pleural fluid variables and underlying diseases.

Animals: Samples of transudates and serum from 18 cats and 37 dogs with transudative effusion (total nucleated cell count [TNCC] <5000 cells/μL) were analyzed.

Methods: Lipoprotein fractions, triglyceride, and cholesterol (CHO) concentrations were prospectively determined in paired fluid and serum samples. Standard fluid measurements were retrospectively collected.

Results: Two distinct fluid lipoprotein patterns were noted. Fluids rich in VLDL+IDL were associated with chronic kidney disease, acquired portosystemic shunts or protein-losing enteropathy (group I). Fluids rich in denser lipoproteins were associated with underlying heart disease, caudal vena cava syndrome or intracavitary neoplasia (group II). Group I and group II also had significant differences between fluid concentrations of CHO ($\tilde{x}$ = 8 vs 110 mg/dL) and TP ($\tilde{x}$ = 0.6 vs 3.8 g/dL), respectively. Five peritoneal transudates were triglyceride-rich (>100 mg/dL) and associated with pancreatitis.

Conclusions and Clinical Importance: Protein-poor (TP <1.5 g/dL) and protein-rich (TP >2.5 g/dL) transudates were associated with distinct lipoprotein patterns and specific groups of disease. Effusions secondary to pancreatitis might be transudative and rich in triglycerides.

KEYWORDS
cavitary effusion, cholesterol, triglycerides, pancreatitis

Abbreviations: ALB, albumin; APSS, acquired portosystemic shunt; CHO, cholesterol; CKD, chronic kidney disease; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; PLE, protein-losing enteropathy; TNCC, total nucleated cell count; TP, total protein; TRI, triglycerides; VLDL, very-low-density lipoprotein.

1 | INTRODUCTION

Documented causes of pleural or peritoneal transudates in dogs and cats include congestive heart disease, chronic liver disease, nephrotic syndrome, protein-losing enteropathy (PLE), vena cava syndrome, and neoplasia, among others.[1-4] However, the pathophysiologic mechanisms responsible for formation of these effusions have not been fully elucidated. Total protein (TP) and total nucleated cell count (TNCC) are used to classify effusions as an exudate, modified transudate or pure transudate and can help determine underlying pathogenesis. However, the primary cause of transudate formation is often unidentified.

Lipoproteins can be divided into very-low-density (VLDL), intermediate-density (IDL), low-density (LDL), and high-density (HDL) lipoproteins, according to their diameter and hydrated density. The measurement of lipoproteins in body fluid (BF) samples has primarily been used to diagnose chylous effusion in both humans and animals.[5-8] In human medicine, other fluid constituents, including lactate dehydrogenase activity and cholesterol (CHO) and albumin (ALB) concentrations, have been used to help characterize cavitary effusions.[9-13]

We hypothesized that distinct lipid and protein patterns exist in effusions from dogs and cats and that these are associated with specific groups of diseases. To test this hypothesis, we analyzed serum and fluid samples from animals with transudative effusions and collected their respective clinical data. Our objectives included determining the lipoprotein profile of transudates in dogs and cats and to correlate these findings with both underlying diseases and standard fluid analysis data.

2 | MATERIALS AND METHODS

This was a prospective cross-sectional/cohort study. Eligible samples were collected over 12 months (from April 2019 to March 2020) to assure that the sample time period was representative and that a wide range of diseases were included. All BF and serum samples were retrieved from the William Pritchard's Veterinary Medical Teaching Hospital, hematology lab, UC Davis.

Inclusion criteria for cases consisted of: Species (cat or dog), all ages, both sexes, and BF TNCC below 5000/μL. The cutoff for TNCC was adapted from a combination of 2 publications.[4,14] For a BF to be included in the study, the animal had to have a pleural or peritoneal fluid analysis and a serum chemistry panel submitted within 24 hours of each other. Serum and BF needed to have a minimum volume of 500 μL. When the same animal had additional samples collected from the same cavity analyzed throughout the period of the study, only the first sample was included.

Exclusion criteria included BF samples that had a RBC count >100 000/μL, as hemorrhage can increase chylomicrons in fluid.

Samples were kept at 4°C for no longer than 6 days after which they were aliquoted into cryotubes and stored at −80°C until analysis (<2 months).

Total protein, ALB, triglyceride (TRI), and CHO concentrations were measured in serum and BF samples per standard laboratory operating procedures. An aliquot of each BF sample was also

TABLE 1 Clinical criteria used to include cases of dogs and cats with peritoneal or pleural transudate in each disease diagnosis

Diagnosis	Criteria
Acquired portosystemic shunt	Gross, histopathologic and/or radiologic evidence of vascular communications between the portal and caval circulations[8,9,15]
Protein-losing enteropathy	History of chronic diarrhea (> 3 weeks), panhypoproteinemia, hypocobalaminemia, and hypocholesterolemia with or without histopathologic evidence of lymphoplasmacytic inflammation and lacteal dilatation. Other potential causes for chronic diarrhea were ruled out[2,16,17]
Chronic kidney disease	Appropriate history, clinical, laboratory and imaging findings including polyuria and polydipsia, vomiting, halitosis, ulcerative stomatitis and gastroenteritis, kidney atrophy, moderate to marked renal azotemia, metabolic acidosis, hyperphosphatemia, hypokalemia (for cats), and nonregenerative anemia[5]
Heart disease	• For abdominal effusions: Appropriate history with presence of cardiac murmur and severe structural right-sided heart disease diagnosed by echocardiography, with or without concurrent cardiac arrhythmia, response to treatment for congestive cardiac disease, with hepatomegaly and histopathologic evidence of hepatic congestion • For pleural effusions: Appropriate history with presence of cardiac murmur and severe structural left-sided heart disease diagnosed by echocardiography, with or without concurrent cardiac arrhythmia, pulmonary edema and response to treatment for congestive cardiac disease, alternatively by the presence of cardiac tamponade
Caudal vena cava syndrome	For abdominal effusions only: Radiologic or histopathologic evidence of thrombotic or malignant disease of the caudal vena cava with concurrent hepatomegaly and hepatic congestion
Neoplastic effusion	Diagnosis of malignancy with histopathology in an organ inside the same body cavity where the effusion developed
Pancreatitis	For abdominal effusions only: Appropriate history, clinical signs, and positive SNAP cPL Test Kit (Idexx Laboratories Inc, Westbrook, MEVetS- can cPL Rapid Test, Abaxis Inc, Union City, CA) or ultrasonographic evidence of active pancreatitis, including altered parenchymal echogenicity and organ size
Thrombotic disease	Gross, histopathologic, and/or radiologic evidence of thrombotic disease
Intracavitary lymph node disease	Gross, histopathologic, and/or radiologic evidence of intrapleural or intraabdominal lymph node disease
Fluid overload	Evidence of crystalloid fluid overload based on medical history and physical examination

submitted for lipoprotein electrophoresis to the clinical pathology laboratory of Cornell University. High resolution polyacrylamide electrophoresis with a CHO avid dye and densitometry were used to generate the area under the curves and establish the proportion of VLDL, IDL, LDL, and HDL in the fluid samples.

Clinical information retrospectively collected from the medical record included basic signalment, as well as basic body fluid variables (ie, total and differential cell counts and TP), clinical diagnosis, diagnostic procedures, history, and imaging findings. Clinical diagnosis was established according to the criteria in Table 1.

TABLE 2 Numbers of dogs and cats with cavitary effusion in each disease diagnosis included in the study

	Dogs (n)		Cats (n)		
Diagnosis	**Abdominal**	**Pleural**	**Abdominal**	**Pleural**	**Total**
Congestive heart disease	11	1	2	1	15
Intracavitary neoplasia	7	1	4	1	13
Chronic kidney disease	3	-	3	3	9
Thrombotic disease	6	1	-	1	8
Acquired portosystemic shunt	6	-	-	-	6
Protein-losing enteropathy	5	1	-	-	6
Pancreatitis	2	-	4	-	6
Intracavitary lymph node disease	2	2	1	1	6
Caudal vena cava syndrome	4	-	-	-	4
Fluid overload	1	-	3	-	4
Total[a]	31	6	13	5	55

Note: Confirmed diagnoses only.
[a]Rows do not add up because cases with multiple diagnoses are included.

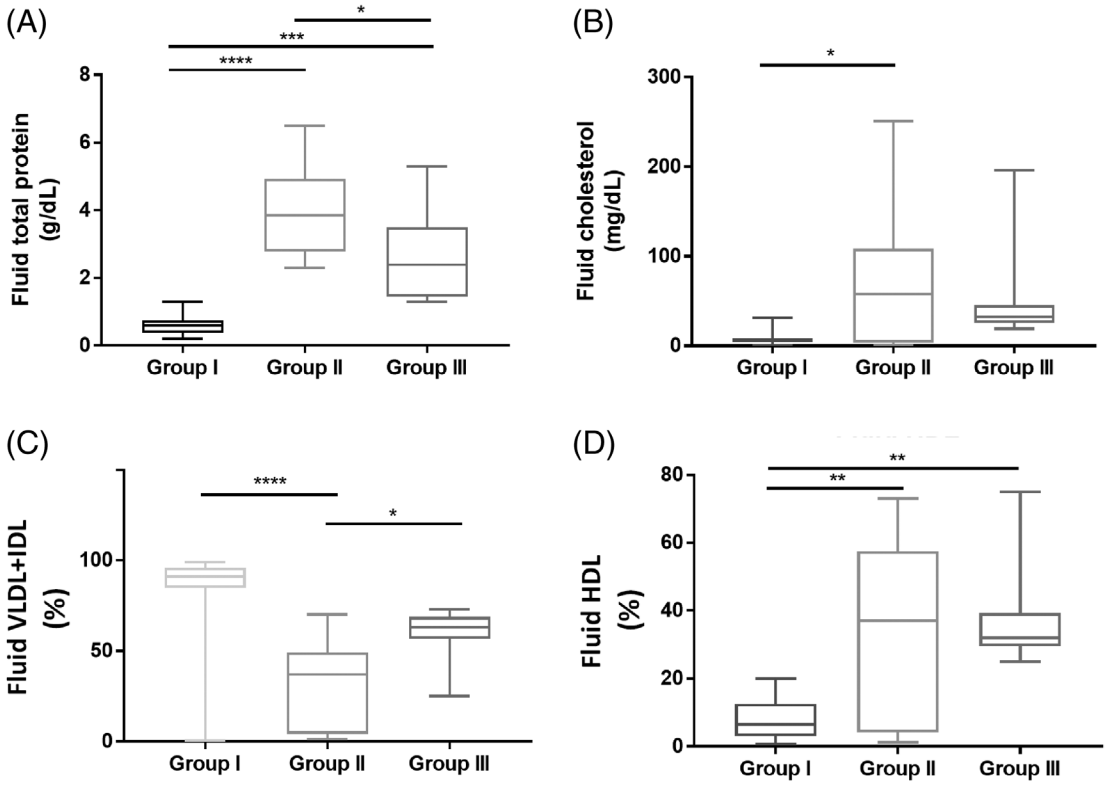

FIGURE 1 Boxplots of the distribution of a few protein, lipid, and lipoprotein variables measured in body fluid samples from dogs and cats with transudate effusion. Bars and asterisks indicate significant statistical differences (Tukey's or Dunn's multiple comparison tests)

2.1 | Statistical analysis

Data were tested for normality using the D'Agostino-Pearson Test. Data that were not normally distributed were analyzed using the Kruskal–Wallis (non-parametric) test. Normally distributed data were analyzed using a 1-way ANOVA. Either Tukey's or Dunn's multiple comparison (post-test) was done as a follow-up to determine significance between groups. A Pearson correlation test was performed among all variables collected.

3 | RESULTS

Fifty-five paired BF and serum samples were included in the study. There were 37 samples from dogs (37/55; 67%) and 18 samples from cats (18/55; 33%). Of these samples, 44 were peritoneal effusions (80%) and 11 were pleural effusions (20%: Table 2). Based on criteria represented in Table 1, 15 cases were associated with heart disease (HD), 13 with intracavitary neoplasia (NEO), 9 with chronic kidney disease (CKD), 6 with acquired portosystemic shunt (APSS), 6 with PLE, and 4 with caudal vena cava syndrome (CVCS). Overall, 17 cases had >1 confirmed or suspected diagnosis. The cases of NEO included lymphoma, carcinoma, intestinal sarcoma, cardiac paraganglioma, and adrenal gland neoplasia.

The percentage of each lipoprotein ranged from 7% to 62% for VLDL, 8% to 59% for IDL, 0% to 35% for LDL, and 1% to 75% for HDL. (Table 3) Two distinct patterns could be recognized based on the proportion of these lipoproteins. The first pattern was rich in VLDL and IDL ($\bar{x}$ >80%) and poor in HDLs ($\bar{x}$ <10%). The second pattern was rich in HDL ($\bar{x}$ >50%) and poor in VLDL and IDL ($\bar{x}$ <45%). By examining which etiologies were associated with these 2 patterns, diseases could be segregated into 2 groups based on the lipoprotein pattern; group I (VLDL+IDL rich) included PLE, CKD, and

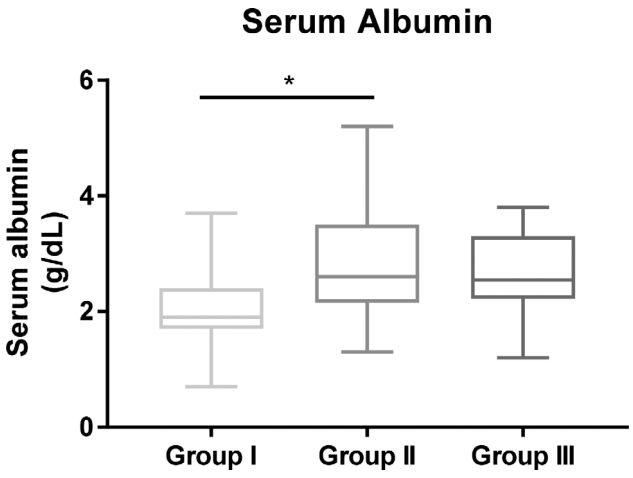

FIGURE 2 Boxplot of the distribution of the concentration of albumin measured in serum samples from dogs and cats with transudate effusion. Bars and asterisk indicate significant statistical differences (Tukey's or Dunn's multiple comparison tests)

TABLE 3 Minimum and maximum (mean and SD) values of fluid and serum variables from 35 dogs and cats with peritoneal or pleural transudate

	Diseases[a]					
	Group I			Group II		
Variables	APSS (n = 4)	CKD (n = 4)	PLE (n = 6)	HD (n = 14)	CVCS (n = 3)	NEO (n = 10)
Fluid						
TP (g/dL)	0.2 to 0.9 (0.6 ± 0.4)	0.3 to 0.7 (0.5 ± 0.2)	0.3 to 1.3 (0.6 ± 0.3)	2.3 to 6.5 (4.0 ± 1.3)	3.5 to 4.9 (4.1 ± 0.7)	2.3 to 6.5 (3.7 ± 1.2)
TNCC (cells/μL)	40 to 540 (240 ± 265)	30 to 920 (264 ± 438)	50 to 1600 (593 ± 759)	160 to 4200 (1295 ± 1150)	520 to 840 (660 ± 164)	240 to 3360 (1057 ± 933)
CHO (mg/dL)	4 to 8 (7 ± 2)	4 to 7 (5 ± 2)	4 to 31 (10 ± 10)	55 to 251 (134 ± 76)	99 to 251 (175 ± 107)	41 to 251 (117 ± 77)
TRI (mg/dL)	23 to 74 (49 ± 26)	9 to 26 (17 ± 9)	18 to 66 (34 ± 18)	36 to 177 (88 ± 54)	45 to 98 (72 ± 37)	36 to 177 (71 ± 45)
VLDL+IDL (%)	89 to 99 (95 ± 5)	91 to 96 (93 ± 3)	81 to 96 (90 ± 6)	15 to 68 (43 ± 15)	36 to 50 (43 ± 10)	26 to 70 (45 ± 16)
HDL (%)	1 to 9 (4 ± 4)	3 to 7 (5 ± 2)	4 to 19 (10 ± 6)	27 to 73 (50 ± 15)	49 to 60 (55 ± 8)	26 to 73 (50 ± 17)
Serum						
TP (g/dL)	4.1 to 5.4 (4.8 ± 0.7)	5.0 to 7.9 (6.1 ± 1.3)	3.0 to 3.9 (3.5 ± 0.4)	5.0 to 7.4 (5.6 ± 0.8)	5.0 to 5.0 (N/A)	4.1 to 7.4 (5.5 ± 1.0)
ALB (g/dL)	2.3 to 2.9 (2.7 ± 0.3)	1.8 to 3.7 (2.5 ± 0.8)	1.6 to 2.4 (1.9 ± 0.3)	2.1 to 4.8 (3.1 ± 0.9)	2.9 to 2.9 (N/A)	2.1 to 4.8 (2.9 ± 1.0)
CHO (mg/dL)	136 to 370 (215 ± 135)	0 to 301 (143 ± 131)	67 to 161 (106 ± 35)	95 to 324 (210 ± 72)	280 to 280 N/A)	109 to 280 (193 ± 68)
TRI (mg/dL)	42 to 154 (82 ± 63)	14 to 70 (44 ± 28)	62 to 84 (72 ± 10)	33 to 290 (115 ± 69)	48 to 48 (N/A)	48 to 385 (140 ± 139)

[a]Numbers on row 3 (ie, "n") do not add up because cases with a concurrent disease inside the same group (but not in different groups) are represented.

Abbreviations: ALB, albumin; APSS, acquired portosystemic shunt; CHO, cholesterol; CKD, chronic kidney disease; CVCS, caudal vena cava syndrome; HD, heart disease; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; N/A, not enough value points to calculate; NEO, intracavitary neoplasia; PLE, protein-losing enteropathy; TNCC, total nucleated cell count; TP, total protein; TRI, triglycerides; VLDL, very-low-density lipoprotein.

APSS; group II (HDL rich) included HD, NEO, and CVCS. These groups formed the basis for downstream analysis. Fourteen cases were associated with diseases from group I (and no other effusive etiology) and 21 with diseases from group II (and no other effusive etiology). A third group (group III) was created, which contained cases concurrently associated with diagnoses from both the first 2 groups (n = 10). Cases in which a definitive diagnosis associated with a typical effusive etiology could not be established (n = 10) were not included in any of the 3 groups. The LDL concentrations were overall much lower than the other lipoprotein subfractions and did not seem to be associated with any specific disease or effusion classification.

Fluid TP was significantly different between all 3 groups ($P < .0001$), with cases from group I exhibiting the lowest values (Figure 1A). Fluid CHO ($P = .01$) and serum ALB concentrations ($P = .03$) were significantly different between groups I and II only (Figure 1B and Figure 2, respectively). Fluid VLDL+IDL were significantly different between groups I and II ($P < .0001$) and II and III ($P = .04$) but not between groups I and III (Figure 1C). Fluid HDL was significantly different between groups I and II ($P = .005$) and I and III ($P < .009$, Figure 1D) only. Fluid TNCC, TRI, and serum TP were not significantly different between any groups. The correlation between the variable TP and the variables CHO, VLDL+IDL, and HDL was significant ($r = .89$, $-.86$, and .91, respectively, Pearson's correlation test).

Even though a five effusions had high TRIs (ie, >100 mg/dL and Tf:Ts >1.0, Supplemental Table 1), no effusion contained chylomicrons. Five out of the 6 cases of TRI-rich effusions were peritoneal in origin and associated with pancreatitis (Supplemental Table 1). The sixth case (case #1) was a pleural effusion associated with a suspected PLE. This effusion was a sample from necropsy and, therefore, a total and differential cell count or the protein levels were not available. We decided to include it in the study as part of the TRI-rich effusions group, but, because we could not confirm it as a transudate, its data was not used in any other type of analysis. These effusions all had a predominance of neutrophils (ie, neutrophil count >50%) and a lymphocyte count below 10%, except for case #6, which had 30% lymphocytes. The overall mean (±SD) values for differential count were 53.5% (±26.6) neutrophils, 13.1% (±14.3) lymphocytes, 31.5% (±24.0) macrophages, and 0.9% (±3.6) eosinophils.

4 | DISCUSSION

We demonstrate that distinct lipid and protein patterns exist in body fluids from dogs and cats and these are associated with specific groups of diseases. Effusions secondary to PLE, CKD, and APSS were poor in protein (<1.5 g/dL), CHO (<20 mg/dL), and HDL. These effusions also had a significantly higher proportion of VLDL and IDL in their lipoprotein profile. Conversely, effusions secondary to heart disease and intracavitary NEO had high TP (>2.5 g/dL), high CHO (>50 mg/dL), and a higher proportion of HDL. Effusions from animals with multiple underlying disease patterns (group III) had BF features, including the lipoprotein pattern, TP and CHO, which were intermediate to both groups.

Dogs and cats appear to have transudative effusions secondary to pancreatitis. These appear to be sometimes rich in TRI and have variable TP and TNCC values. Thrombotic underlying etiologies and vena cava obstructive disease, including adrenal gland neoplastic invasion, might represent overlooked causes of protein-rich (and CHO-rich) transudates.

Since TP was significantly different between groups, and correlated either positively or negatively with CHO, VLDL+IDL, and HDL, and TNCC was neither significantly different between groups nor correlated with other variables (Table 3), we propose a new diagnostic algorithm, as follows: Effusions with TNCC >5000 should be classified as exudates, regardless of TP. Effusions with <5000 TNCC should be subdivided based on their TP, using a cutoff of <1.5 g/dL to suspect PLE, CKD, or APSS and >2.5 g/dL to suspect HD, CVCS, or NEO. For cases with intermediate TP values, a complex case with multiple etiologies, or a less common etiology, should be suspected.

The lipoprotein profile of human pleural transudates and exudates showed that inflammatory and malignant pleural effusions were rich in LDL, and that pseudochylous effusions were rich in HDL.[6,18] Similar to findings in our study, transudates were also found to be poor in LDL and HDL. A relationship between low fluid CHO and a transudative etiology has been previously established.[9-11,15,16,19,20] In the current study, the paucity of the CHO-rich LDL and HDL lipoproteins in group 1 animals aligns with their low CHO concentration whereas group 2 (HDL-rich) has the anticipated higher CHO.

All 5 peritoneal effusions that were TRI-rich, chylomicron negative were associated with moderate to severe acute pancreatitis, diagnosed according to criteria displayed in Table 1. The most current and precise diagnostic guidelines for a chylous effusion in human medicine involve a fluid with concentrations of TRI > 110 mg/dL, CHO < 200 mg/dL, presence of chylomicrons and absence of CHO crystals.[21] However, a TRI-rich effusion in both human and veterinary medicine practices is traditionally considered synonymous with chylous effusion. These TRI-rich, chylomicron negative effusions from our study were not classically chylous and cytologically were not characterized by a predominance of lymphocytes. Some of these TRI-rich peritoneal effusions had a hazy to opaque supernatant, typical of chylous effusions.

A few cases of chyloperitoneum secondary to pancreatitis have been reported in the human literature.[17,22-36] Interestingly, none of these publications reported measuring chylomicrons and the pathophysiology of this association is not yet fully elucidated. Among the hypothesized mechanisms of chylous ascites formation in pancreatitis are the direct lymphatic damage by the enzyme-rich fluid, extrapancreatic necrosis, compression of the lymphatics by inflammatory changes, and surgical injury. Severe acute pancreatitis is also often associated with mesenteric fat necrosis caused by release of pancreatic enzymes into the peritoneal cavity.[37,38] We speculate that such a process could induce release of TRIs into the cavity, after the enzymatic lysis and rupture of adipocytes in the mesentery. Chylomicrons are only used to transport TRIs in the circulation and are not found within adipocytes. Therefore, the resulting effusion, in this scenario, would be expected to be TRI-rich, but chylomicron-free. Traditionally,

this process was thought to elicit an inflammatory response strong enough to induce formation of an exudate, by increased vascular permeability. We further speculate that, perhaps, it is possible the formation of a transudate might be associated with the development of parenchymal edema; another classic histopathologic feature of acute pancreatitis.[39] In a study with dogs, 43% of cases of peritoneal effusion secondary to pancreatitis were transudates.[40] The authors recommend that, if a peritoneal effusion is rich in triglycerides, but not predominated by lymphocytes and not associated with any obvious chylous etiology, further investigation to rule out underlying pancreatitis should be warranted.

The 1 case with a TRI-rich pleural effusion was a dog, for which no apparent underlying chylous etiology was detected on necropsy (case #1, Supplemental Table 1). However, this dog did have severe pancreatitis with marked fat necrosis noted on necropsy. It has been postulated that hydrothorax can form in human cirrhotic liver disease animals by the transfer of ascitic fluid between cavities because of a combination of thoracic negative pressure and microscopic diaphragmatic fenestrations.[41] Studies involving dogs and other animal species have not been conducted, but it is possible that the TRI-rich pleural transudate described in this study might have formed because of ascitic fluid translocation. Pleural effusion secondary to either acute or chronic pancreatitis affects 20% to 50% of human animals with these conditions.[42-44] The pleural effusion pathogenesis is still unknown but speculated to be related to lymphatic spread of fluid across the diaphragm.[45] It is also possible that this was an idiopathic chylothorax; however, the lack of chylomicrons in the fluid does not support this hypothesis.

Another proposed pathophysiologic mechanism to explain the association between chylous effusion and pancreatitis is the pre-existence of a condition leading to chylomicronemia.[25] Hypertriglyceridemia is described as a predisposing factor for the development of pancreatitis in small animals.[46] Chylomicrons, the largest of the lipoprotein molecules, can block the normal pancreatic microcirculation and also lead to activation of trypsinogen into trypsin leading to inflammation and destruction of the acinar parenchyma.[47] In an animal with hypertriglyceridemia, an effusion caused by increased vascular hydrodynamic pressure of any nature (and not specifically or directly associated with pancreatitis) would have the potential to elicit a triglyceride-rich effusion because of plasma transfer (and not chyle leakage). For our animals, a transient condition of hypertriglyceridemia/chylomicronemia cannot be ruled out. However, there was no clinical suspicion of disease secondary to hypertriglyceridemia and, even though not every animal had serum TRI measured, the ones that did, had a lower concentration compared to fluid TRI (Supplemental Table 4).

Transudative chylous effusions have been reported many times in human animals. They are defined as an effusion with transudative features, including being physically clear with low TP and LDH values, but in which chylomicrons are detected through LPE.[48] These fluid accumulations have been associated with cirrhosis, nephrotic syndrome, vena cava thrombosis and pulmonary hypertension.[48-51] We did not detect transudative chylous effusions in the present study. Nevertheless, results from this study show that TRI-rich effusions can be poor in protein and cellularity.

Additional pathophysiologic mechanisms that could explain effusion formation among the cases selected in our study include post-sinusoidal portal hypertension. Hepatomegaly, hepatic congestion, or both abnormalities were identified in many of the cases associated with HD and CVCS, which support the assumption that this was the major mechanism playing a role in fluid accumulation in these cases. Perhaps, other larger molecules, including CHO-rich lipoproteins (ie, HDL and LDL), are also able to leave the circulation secondary to post-sinusoidal portal hypertension, explaining part of the findings in this study. Indeed, a few studies have verified a correlation between increased pleural capillary permeability and a higher concentration of CHO in pleural effusions.[52,53]

Lipoprotein electrophoresis was also performed in a subset of serum samples with the purpose of verifying if lipoproteins in fluid were just a reflection of the contents of the blood or if there were independent factors affecting fluid profiles. Table 3, however, shows how TRI and CHO levels were different between fluid and serum samples, especially among group I samples. Even though serum levels of these 2 analytes from any moment previous to sampling could have been different, we presume that lipoprotein contents in fluid are likely not just a mere reflection of blood content of these molecules. Studies in humans have shown robust changes in lipid metabolism in both the thoracic and peritoneal cavities in a number of disease states, supporting the hypothesis that intracavitary metabolism can impact lipoproteins in effusions.[8,18,52,54,55]

Intracavitary NEO can compress vessels and produce a transudate through increased vascular hydrodynamic pressure and through irregular neovascularization.[56,57] All cases of CVCS also had intracavitary NEO (adrenal gland neoplasia invading the caudal vena cava). Differentiating between the role of intracavitary NEO and CVCS in the effusive process was not possible in this study.

Serum ALB concentration was lower among cases in group I compared to group II (Figure 2). In veterinary medicine, hypoalbuminemia alone rarely results in effusion formation.[14,58-60] One study found that ascites is more frequent in dogs with acquired PSS, rather than dogs with congenital PSS, even though no significant difference in the serum ALB between the 2 groups of dogs was noted.[61] Human studies have elucidated the importance of sodium and water retention as key players in ascites formation secondary to cirrhosis or end-stage liver disease.[62-65] Serum ALB concentration also might have been lower for group I cases because of third-space loss of ALB.

Lymphatic hypertension can lead to formation of a low-protein effusion because of accumulation of normal body fluid, which is typically poor in protein,[3,66] from reduced removal through lymphatics.[67] If this was the pathogenesis for some of the protein-poor transudates found in this study, our findings might support that VLDL and IDL are the lipoproteins found in normal body fluids from dogs and cats. Two cases in group II had a VLDL+IDL percentage higher than average for that group (making their lipoprotein pattern more similar to that seen in group I). These cases were associated with intracavitary nodal lymphoma and lymphatic hypertension could have contributed to the effusion. Lymphangiectasia is a commonly associated finding with PLE.[68] It

is possible that lymphatic hypertension, and not hypoalbuminemia, was the cause of effusion in cases of PLE with protein-poor transudates.

The decision to use only TNCC as an inclusion criterium (and not TP) allowed a number of cases with a wider range of fluid TP to be included in the study. Interestingly, all cases with higher TP values were associated with transudative diseases (eg, HD and NEO) and had similar lipoprotein pattern. Our data support not differentiating transudates from exudates by protein alone.

Based on results of this study, lipoprotein electrophoresis analysis correlates really well with TP, a measurement already widely used to classify effusions. Such finding contributes to avoid the need to appeal to an expensive, time-consuming, and poorly available technique, such as LPE, to figure out what is causing fluid accumulation in dogs and cats. In other words: lipoprotein analysis does not seem to help much more than TP and results of this study did not really support its use in clinical practice. However, if both TP and lipoprotein analysis are performed and there is discordance between TP and lipoprotein patterns (or results do not fit with what disease an animal is known to have) that could support the presence of additional/more complicated disease processes. Cases in group III (ie, those included in the study with >1 type of underlying etiology), however, presented lower correlation values between these variables ($r = .72$, -0.68, and .71 for CHO, VLDL+IDL, and HDL, respectively), which might support the importance of screening for additional etiologies when inconsistencies between these 2 groups of variables are found.

This study included a number of limitations. The diagnoses were all obtained retrospectively; and therefore, it was impossible to fully determine the underlying etiology responsible for effusion formation. Many animals had multiple concurrent diseases, which also prohibited clear delineation of mechanisms of effusion formation. Animals also had concurrent non-effusive etiologies that could impact serum or fluid measurements. Cases in which the transudate was thought to have originated from pancreatitis would have benefited from having amylase and lipase measured in serum and fluid to verify that pancreatitis was the underlying cause of effusion formation. Some diagnoses (eg, pancreatitis) could not be fully excluded in all cases, because these cases often present with nonspecific clinical signs and a full diagnostic work-up is not always pursued.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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