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Comparative pathophysiology and management of protein-losing enteropathy

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Protein-losing enteropathy, or PLE, is not a disease but a syndrome that develops in numerous disease states of differing etiologies and often involving the lymphatic system, such as lymphangiectasia and lymphangitis in dogs. The pathophysiology of lymphatic disease is incompletely understood, and the disease is challenging to manage. Understanding of PLE mechanisms requires knowledge of lymphatic system structure and function, which are reviewed here. The mechanisms of enteric protein loss in PLE are identical in dogs and people, irrespective of the underlying cause. In people, PLE is usually associated with primary intestinal lymphangiectasia, suspected to arise from genetic susceptibility, or "idiopathic" lymphatic vascular obstruction. In dogs, PLE is most often a feature of inflammatory bowel disease (IBD), and less frequently intestinal lymphangiectasia, although it is not proven which process is the true driving defect. In cats, PLE is relatively rare. Review of the veterinary literature (1977-2018) reveals that PLE was life-ending in 54.2% of dogs compared to published disease-associated deaths in IBD of <20%, implying that PLE is not merely a continuum of IBD spectrum pathophysiology. In people, diet is the cornerstone of management, whereas dogs are often treated with immunosuppression for causes of PLE including lymphangiectasia, lymphangitis, and crypt disease. Currently, however, there is no scientific, extrapolated, or evidence-based support for an autoimmune or immune-mediated mechanism. Moreover, people with PLE have disease-associated loss of immune function, including lymphopenia, severe CD4+ T-cell depletion, and negative vaccinal titers. Comparison of PLE in people and dogs is undertaken here, and theories in treatment of PLE are presented.

KEYWORDS

crypt, hypoalbuminemia, lymphangiectasia, lymphatic, panhypoproteinemia, PLE

Abbreviations: APCs, antigen presenting cells; ATIII, antithrombin III; BECs, blood endothelial cells; CCECAI, canine chronic enteropathy clinical activity index; CD, Crohn's disease; CE, chronic enteropathy; CIBDAI, canine IBD activity index; CRP, C-reactive protein; CT/MRI, computerized tomography/magnetic resonance imaging; EFA, essential fatty acid; α1 PI, alpha 1 protease inhibitor; FISH, fluorescence in situ hybridization; GALT, gut-associated lymphoid tissue; GI, gastrointestinal; GL, granulomatous lymphangitis; HS, heparan sulfate; IBD, inflammatory bowel disease; IL, intestinal lymphangiectasia; IMPDH, inosine monophosphate dehydrogenase; IP, immunophenotyping; LCFA, long-chain fatty acids; LECs, lymphatic endothelial cells; LN, lymph node; MCFA, medium-chain fatty acids; MCT, medium-chain triglycerides; PARR/PCR, PCR for antigen-receptor rearrangements; PIL, primary intestinal lymphangiectasia; PLE, protein-losing enteropathy; PLN, protein-losing nephropathy; RER, resting energy requirement; PTE, pulmonary thromboembolism; SCFA, short-chain fatty acids; SCWT, Soft Coated Wheaten Terrier; SI, small intestinal; TE, thromboembolism; UC, ulcerative colitis; UFH, unfractionated heparin sulfate; YT, Yorkshire Terrier; YT-PLE, Yorkshire Terrier protein-losing enteropathy.

1 | COMPARATIVE PATHOPHYSIOLOGY OF PROTEIN-LOSING ENTEROPATHY

In dogs and people, protein-losing enteropathy (PLE) is associated with numerous related or unrelated diseases (Table 1), but there are a limited number of mechanisms by which plasma protein is lost through the gastrointestinal (GI) tract[1–3]:

- Physical or functional lymphatic obstruction leading to "overflow" lymph leak, for example, congenital or acquired lymphatic disease.
- Release of cellular mediators affecting vascular permeability and causing fluid egress into tissues, for example, widespread mast cell activation, eosinophilic gastroenteropathy.

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TABLE 1 Causes of PLE in people and dogs

People	Dogs
1. Mucosal injury	
a. Erosive	
Inflammatory bowel diseases (Crohn's disease, ulcerative colitis)	Inflammatory bowel diseases (lymphoplasmacytic, eosinophilic, granulomatous)
Infections: *Giardia, Clostridium, Campylobacter, Salmonella, rotavirus, Whipple's disease*, intestinal tuberculosis	Infections: *Parvovirus, Clostridium, Campylobacter, Salmonellosis, Histoplasmosis, Schistosomiasis (Heterobilharzia americana)*
Neoplasia	Neoplasia
Nonsteroidal enteropathy	Nonsteroidal enteropathy
b. Non-Erosive	
Menetrier's disease (hypertrophic gastritis)	Diet-induced enteropathy
Eosinophilic gastritis	Immunoproliferative enteropathy
Celiac disease	Hypoadrenocorticism
Lactose or other food intolerance	Intestinal crypt disease
Systemic lupus erythematosus	
Intestinal crypt disease	
2. Infectious	
Lymphatic filariasis	Lymphatic filariasis (RARE)
	Hookworms
	Strongyloides stercoralis
3. Lymphatic disease	
Idiopathic primary IL (Waldmann's disease)	Idiopathic primary IL
Secondary IL: Crohn's disease, neoplasia sarcoidosis, congestive heart failure, restrictive pericarditis	Secondary IL: IBD, neoplasia, lymphatic infections, right-sided congestive cardiac failure
Fontan surgery	Lymphangitis (granulomatous/inflammatory)
Genetic: Lymphodysplasia (Hennekam's syndrome)	
Lymphangitis	

Abbreviations: IBD, inflammatory bowel disease; IL, intestinal lymphangiectasia.

- Mucosal inflammation (nonerosive or erosive/ulcerative), for example, inflammatory bowel disease (IBD).

In each mechanism, protein-rich fluid accumulates in the interstitium and passes into the GI tract via mucosal tight junctions, without requiring mucosal epithelial disruption or ulceration (Figure 1).[3,4] Albumin is the most affected protein in people because of its slow turnover rate, which is why protein loss in PLE is diagnosed by detection of hypoalbuminemia.[5] As a water-soluble protein in dogs and people, albumin maintains plasma oncotic pressure and transports hormones, fatty acids, ions, and bilirubin. In health, enteric losses in people occur as sloughed enterocytes and normal secretions, and account for around only 1-2% of the entire protein pool and less than 10% of total albumin.[6] In steady state, albumin broken down approximates albumin synthesized in people, but in PLE, protein wasting can amount to 60% of the total protein pool.[7,8] The most severely diminished are long

half-life proteins with limited ability to respond rapidly—albumin, immunoglobulins, and ceruloplasmin.[5] Hepatic synthesis in PLE is normally accelerated so that proteins with shorter half-lives such as IgE, clotting factors, prealbumin, and transferrin are preserved near normal.[1,5]

Protein-losing enteropathy can be asymptomatic but can also present as a life-threatening condition with effusions, lymphedema, and in people, severe disfigurement. Immune defenses are disrupted because of loss of lymphocytes, globulins, iron, calcium, and other serum components (lipids, fat soluble vitamins).[2,4,5,7,8]

The immunological deficits documented in PLE are numerous in people, including impaired antibody function, B-cell depletion, diminished IgG, IgA, and IgM, and T-cell depletion, especially CD4$^+$ T cells.[2] Despite profoundly low CD4$^+$ counts, opportunistic infection appears to be uncommon in people.[9,10] Similar studies are lacking in dogs, but depletion in these key components is to be similarly expected.

Sudden death caused by thromboembolism (TE) occurs in PLE and can manifest in dogs that have clinically "silent" disease.[11] The mechanism is not well defined, but suggested causes of PLE-associated thrombosis include systemic inflammation, altered vitamin K absorption, loss of antithrombin III (ATIII), hyperaggregation of platelets, hyperfibrinogenemia, and vascular compromise.[11–13]

2 | LYMPHATIC STRUCTURE AND FUNCTION

The lymphatics are a unidirectional vascular network forming an extensive drainage system throughout the body with roles of extracellular fluid homeostasis, fat absorption and transport, and immune system function.[3] Running parallel with the venous system, the lymphatic system is broadly divided into lymphatic capillaries (where interstitial fluid enters), prenodal and postnodal vessels, and collecting vessels.[3] Collecting vessels drain to the thoracic duct via discrete regional lymph nodes (LNs) before joining the confluences of large veins.[3,10] Specialized lymphatic endothelial cells (LECs), with structural similarity to blood endothelial cells (BECs), form lymphatic capillary walls. Derived embryologically from the outbranching of cardinal vein BECs, LECs are under control of genes such as Prospero Homeobox Protein 1, Lymphatic Vessel Endothelial Receptor 1, Podoplanin, and Vascular Endothelial Growth Factor 3.[14–18]

2.1 | Lymphatic capillaries

The LECs of capillary walls are not tightly joined, but only the cell edges overlap, forming "flap-like" valves (Figure 1).[19] Lymphatic endothelial cells have no support (mural) cells but attach to the extracellular matrix via fine collagen filaments that anchor to surrounding tissues. This allows LECs to operate like swinging doors, receiving interstitial fluid in a strictly 1-way system.[3] When pressure in the lymphatic capillary exceeds interstitial pressure, the endothelial "door" flap remains shut, preventing lymph from leaving.[3,20] But when interstitial pressure exceeds lymphatic capillary pressure, the "doors" flap open and interstitial fluid moves to the lymph system.[20] Not only fluid but also other substances such as proteins and pathogens, inflammatory and neoplastic
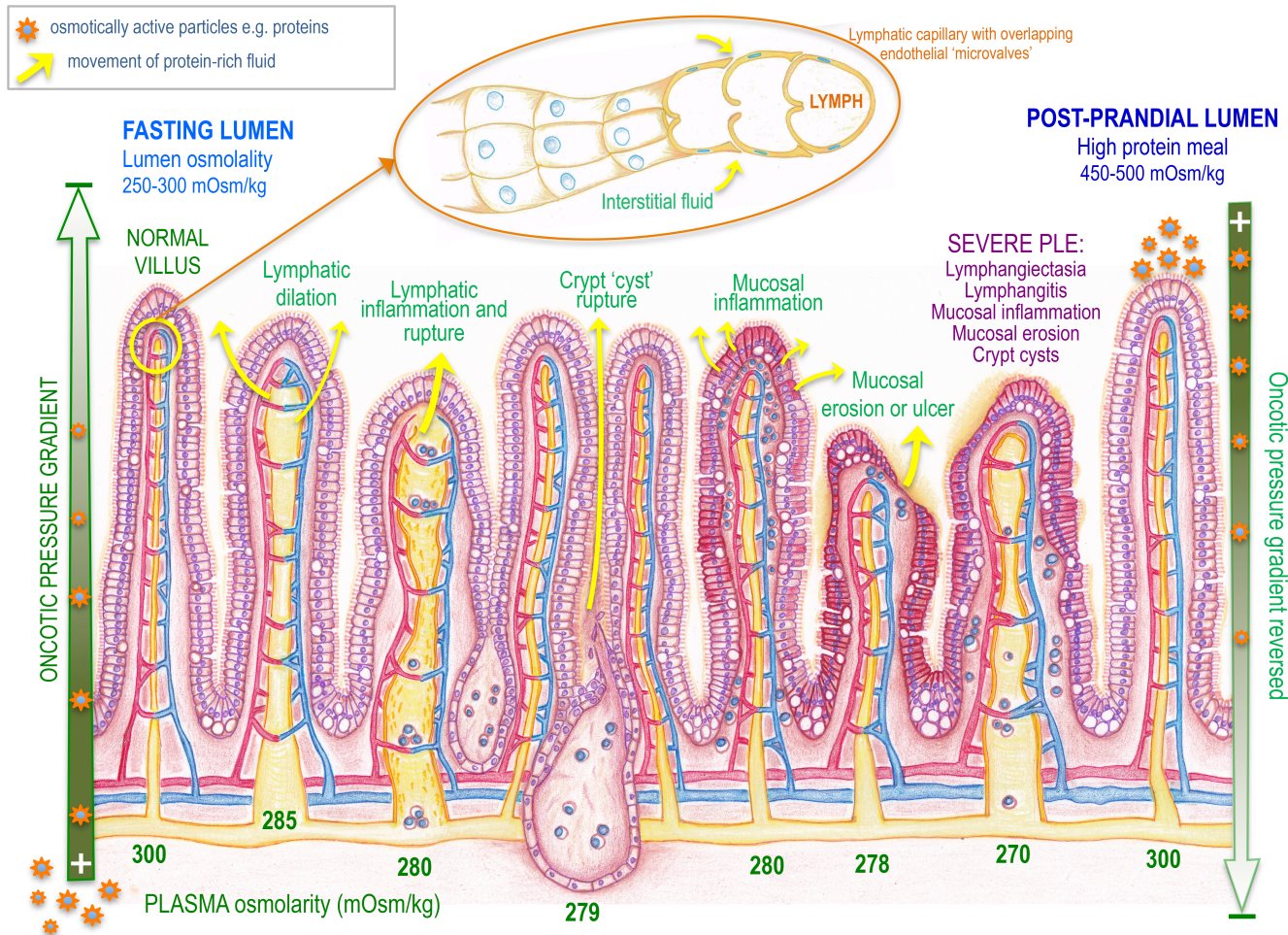
MECHANISMS OF PROTEIN LOSS IN PLE

FIGURE 1 Diagrammatic representation of "PLE syndrome" diseases in dogs (lymphangiectasia, lymphangitis, crypt lesions, IBD, mucosal ulceration) and the effect of Starling's forces on intestinal osmolality and the interstitial-lumenal oncotic gradient. Protein leaks into the small intestine passively, via an oncotic gradient from the interstitium to the lumen. If osmotic pressures equilibrate, a reduction in passive leak is expected. When the intestinal lumen has a higher oncotic pressure than the interstitium, such as postprandially, the gradient may be reversed, depending on the underlying pathology. Top: Magnification of lymphatic capillary structure, showing overlapping endothelial cells that act as flap-like doors allowing interstitial fluid to enter while preventing lymph exit. IBD, inflammatory bowel disease; PLE, protein-losing enteropathy

cells can join lymph in this manner.[21,22] Interstitial proteins enter lymph capillaries easily, despite being too large to enter blood capillaries.[22]

2.2 | Collecting lymphatics and lymphangions

Lymphatic capillaries merge and drain into precollecting vessels. The spindle-shaped LECs of precollectors are continuously adherent, with "zipper"-like junctions.[23] Intraluminal valves prevent backflow of lymph, and smooth muscle cells enable intrinsic contractility.[20,21,24] Precollectors merge into larger collecting vessels, made up of a series of subunits called "lymphangions" (the section between 2 intraluminal valves).[24,25] The collecting vessel wall is organized into a layer of continuously adherent LECs (intima), a basement membrane comprising laminin and type IV collagen, and circumferential smooth muscle (media).[26,27] Lymphangions act individually and in groups, forming contractile units that propel lymph via intraluminal valves made of connective tissue covered with LECs.[23,25,26]

2.3 | Intestinal lymphatic networks

The microanatomy of the intestinal lymphatics is described in ultra-structural studies, and in dogs and people there are 3 layers: central villus lymphatics (lacteals), submucosal lymphatics, and smooth muscle lymphatics.[19,27] The lacteals connect to the submucosal lymphatic network with abundant interconnections. Injection of tracers shows free flow of fluid throughout the submucosal network and into adjacent villi, suggesting a valveless system lacking in smooth muscle and spontaneous contraction.[20] The lymphatic vessels draining the muscle wall form a separate and noncommunicating network. Both networks transport lymph to collecting lymphatics[24] with 1-way valves and spontaneous contractions propelling lymph away from the intestine to the mesentery.[3,20]

2.4 | Tissue fluid homeostasis

Lymphatic capillaries maintain tissue fluid homeostasis by absorbing and transporting extravasated fluid to the venous circulation. Starling

demonstrated that lymphatics play an important role in the regulation of hydrostatic and oncotic pressure throughout the entire body.[28] From the capillary blood, fluid filters continuously into the interstitium and a dynamic equilibrium is reached between filtration, reabsorption, and tissue fluid clearance by lymphatics.[3] The extravasation of fluid is dictated by Starling forces, and in people there can accumulate up to 8 L during 24 hours.[21] Traditionally, venous reabsorption was thought to clear extravasated fluid, but there is now comprehensive published scientific evidence[21,22,24,28] that venous reabsorption is nonexistent in most vascular beds during steady-state conditions.[21,22] Lymph transport is both passive and active. Extrinsic factors such as arterial pulsation, skeletal muscle contraction, and respiration cause passive compression and expansion of the small capillary lymphatics. Intrinsic spontaneous contractions are generated in larger vessels, and in people intraluminal lymph pressure can reach up to 100 mm Hg.[21] Lymphatic vessel contractions are influenced by many different stimuli: humoral, neuronal, pressure, temperature, and shear stress.[21,24] They show reactivity, either inhibitory or stimulatory, to a vast number of vasoactive substances such as substance P, nitric oxide, noradrenaline, histamine, endothelin, and prostaglandins.[21,24,25] Despite evidence of lymphatic innervation, most consistently described as sympathetic, the exact role of the nerve supply in lymphatic function is unclear.[3]

The drainage function of lymphatic vessels is crucial for resolving inflammatory reactions in the interstitium, and during inflammation, lymphatics can proliferate through lymphangiogenesis to remove debris directly or via phagocytic cells.[3,17,21] Development and proliferation of lymphatic vessels is primarily mediated by vascular endothelial growth factor C in pathological states.[29]

2.5 | Lymph nodes

The LNs, spleen, and other secondary lymphoid organs (eg, intestinal Peyer's patches) are anatomically located to capture foreign antigen and antigen presenting cells (APCs) for immune surveillance.[30] Afferent lymphatic vessels traverse the fibrous capsule of the LN and drain lymph into the subcapsular sinus and cortex. The cortex has a paracortex, populated by T cells (for antigen presentation by dendritic cells), and a germinal center, comprised B cells (for humoral immune responses). Lymph drains through the cortex via trabecular sinuses and into the central medullary region via medullary sinuses. The systemic blood circulation interacts with the LN via high endothelial venules in the cortex.[25,31] Lymph then drains from the medulla toward the hilus and exits the LN via an efferent lymph vessel, effectively clearing and filtering extravascular fluid from tissues throughout the entire body.

2.6 | Immune surveillance

Lymphatic vessels function in immune surveillance as the major conduit of antigens and immune cells from the periphery, through the tissues of the immune system. The highly organized microarchitecture of LNs hosts peripheral APCs to meet and inform naive T cells.[25,32] The largest immunologic organ is the GI tract, where immunologic barrier function plays a vital role in maintaining homeostasis. The gut-associated lymphoid tissue (GALT) consists of the intestinal lamina propria cells, intraepithelial lymphocytes, Peyer's patches, and mesenteric LNs.[33] Lymphocytes continuously recirculate from the bloodstream into intestinal lymph through GALT, to exert immune control in almost all tissues.[34] Mature lymphocytes migrate from blood to tissues, and back to the bloodstream via lymph, once or twice a day, until the cell either finds its antigen or is removed by apoptosis.[35]

2.7 | Intestinal lymphatics

Dietary fat is absorbed into lacteals (Figure 1) and transported as chyle to the bloodstream. Resin casts of canine small intestinal (SI) lymphatics have been produced using methacrylate resin and imaging by scanning electron microscopy.[19] The casts show the lymphatic canal of the jejunum as a single, blind-ending, central tubule, with circular constrictions at the villus tip, and oval indentations corresponding to impressions by endothelial nuclei (Figure 2). The central lacteals of the ileum in dogs are smaller, slender, leaf-like shapes and drain to the lymphatic plexus, above the muscularis layer.[19]

2.8 | Fat absorption

The lacteals absorb essential fatty acids (EFAs) from the bowel. Most ingested fat in a normal diet is taken up by the lacteals and transported to the venous circulation.[36] Fatty acids are classified as short-chain, (SCFAs, <6 carbons), medium-chain, (MCFAs, 6-12 carbons), or long-chain fatty acids (LCFAs, 12 carbons).[36] Most dietary fat is in the form of LCFAs and is absorbed by the enterocyte to form chylomicrons, which enter lymph via the lacteal. In addition to lymphatic absorption, medium-chain triglycerides (MCTs) are water soluble and can pass through enterocytes into the portal blood, bypassing the lymphatics.[37] In people and dogs, MCTs are more efficiently digested and absorbed in this way, and the resulting MCFAs are transported directly to the liver to be metabolized.[36–39]

2.8.1 | PLE in people

In people, PLE is usually caused by primary intestinal lymphangiectasia (PIL),[3] described in 1961 by Dr. Thomas A. Waldmann's 18 cases of "idiopathic hypercatabolic hypoproteinemia."[4] These patients had edema associated with hypoproteinemia, low serum albumin, and serum gammaglobulins. The total exchangeable albumin pool, assessed with radiolabeled ^{131}I-albumin, was low in all patients, and daily fecal excretion of ^{131}I was twice that of healthy controls. Intestinal histology showed dilatation of lymph vessels in the mucosa and submucosa and the authors proposed the term "intestinal lymphangiectasia" (IL). Known since as Waldmann's disease, PIL can also be asymptomatic.[40]

The tortuous and dilated lacteals of the mucosa and submucosa in PIL continually leak protein-rich lymph into the bowel. Patients usually present in childhood with edema, weight loss, diarrhea, and lymphopenia with no obvious cause. Edema is pitting because oncotic pressure is low, and moderate serous effusions (peritoneal, pleural, pericardial) are common.[40,41] The worldwide incidence of PIL in people is unknown, but familial forms are rare and there is no predilection for sex or race.[2,40] Pathophysiology is unclear, but theories include genetic anomaly and lymphatic obstruction.[42] Lymphatic obstruction theory says that lymphatic hypoplasia or idiopathic malformation causes obstruction
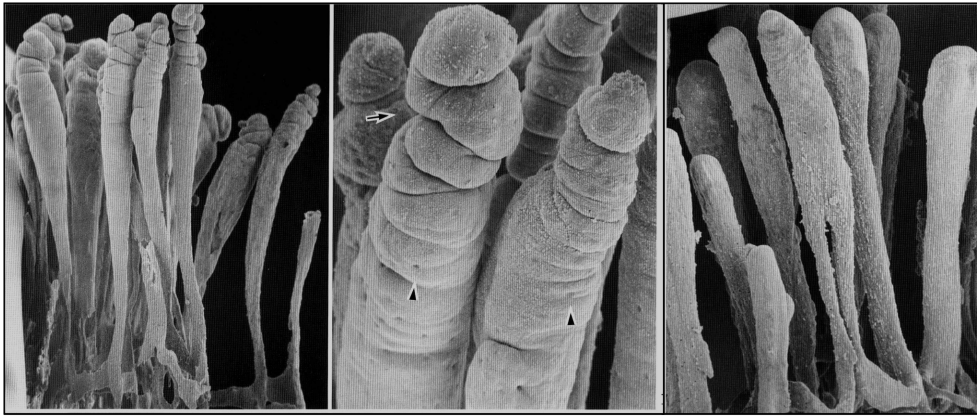
FIGURE 2 A scanning electron microscopy view (left) of a corrosion cast of central lacteals in canine jejunum. A higher magnification (center) showing circular constrictions (arrow) near the top of the lacteals. Arrowheads show imprints of endothelial nuclei. Central lacteals in the ileum (right) are slender leaf-like forms. ×110. From Yamanaka et al.,[23] with permission

and pressure-induced rupture of cystically dilated lymphatic vessels.[42] Genetic theory implicates genes with vital roles in lymphogenesis, that is, VEGF3, prospero-related homeobox-transcriptional factor, forkhead transcriptional factor, and *SOX18*.[43] Mutation of *CCBE1*, a gene that produces a protein with functions in extracellular matrix remodeling and migration, is known to cause generalized lymphatic dysplasia (lymphedema and lymphangiectasia) in Hennekam syndrome, an inherited autosomal recessive disorder.[44]

Secondary IL occurs when a primary disease process obstructs lymphatic vessels or when increased venous pressure induces lymphatic hypertension (Table 1). In people, secondary IL has been associated with constrictive pericarditis, lymphoma, Whipple's disease, sarcoidosis, intestinal tuberculosis, and Crohn's disease (CD).[2,40] In CD, numerous studies describe lymphangitis, lymphangiectasia, lymphatic bacterial infiltration, and LN infection.[45] Some authors suggest that the core driving pathology in CD is lymphatic disease, with secondary vasculitis and transmural inflammation.[46–48] In other diseases where PLE occurs, lymphatics are not the root cause and protein is lost via mucosal epithelium by intercellular leak or exudation,[2] for example, eosinophilic gastroenteropathy, Menetrier's disease, autoimmune enteropathy, and systemic lupus erythematosus.[40]

2.8.2 | PLE in dogs

In contrast to people, PLE in dogs is usually associated with lymphoplasmacytic enteritis (LPE) rather than PIL (Table 1). In the last 30 years, published data on PLE includes 23 articles, spanning from 1977 to 2018 (Table 2).[12,13,49–69] Taken together, they describe in total 469 dogs of 61 different breeds, most prevalent the Yorkshire Terrier (YT), Border Collie, German Shepherd, and Rottweiler (Figures 3 and 4). Historical features commonly described are ascites, vomiting, diarrhea, weight loss, polyuria and polydipsia, anorexia, weight loss, and lethargy. Clinical examination findings commonly include abdominal distension (ascites), cachexia, muscle wasting, weakness, depression, dyspnea/tachypnea, and abdominal pain. Clinical scoring systems including CIBDAI[70] (canine IBD activity index) and CCECAI[71] (canine chronic enteropathy clinical activity index) were compared in 8 studies and did not always concur: CIBDAI scores indicated "*mild IBD*" (score 4-5) or "*severe IBD*" (score ≥ 9), whereas CCECAI scores indicated "*clinically*

insignificant disease," (score 0-3) "*moderate disease*"(score 6-8) or "*very severe disease*" (score ≥ 12).

The cumulative median serum albumin level, reported in 21 of 23 studies, was 1.49 g/dL, globulins 1.95 g/dL, and total serum proteins 3.35 g/dL (Figure 5), and low serum cobalamin was reported in 8 of 23 studies.[13,52,54,56,64,66,67,69] Small bowel diseases were LPE, in 319 of 469 dogs (68%) and lymphangiectasia in 214 dogs (58%, Figure 3). Inflammatory lymphatic disease, including lipogranulomatous foci and lymphangitis, was present in 38 dogs (8%). Crypt disease was present in 34 dogs (7.2%). Histology was scored according to the standards of the World Small Animal Veterinary Association (WSAVA) GI Standardization Group[72] in 6 studies, with detailed scores published in 3 (Figure 6).

Treatment was described in 16 of 23 reports.[49,51–57,59,61,62,64–67,69] An immune-mediated cause was nearly always presumed, as shown by the use of immune suppressive medication in 15 of 16 publications (Figure 7).[49,51–57,59,61,62,64–67,69] Prednisolone was most frequently used (15 studies) followed by azathioprine (9 studies), cyclosporine (9 studies), chlorambucil (4 studies), and methotrexate (1). Metronidazole and other antimicrobials were each prescribed in 8 studies. Dietary change to low-fat, limited antigen, hydrolyzed, or home-cooked food was implemented in 14 studies and MCT provision in 2.

Median survival times, reported in 8 studies, were highly variable (months: 1.0, 2.2, 5, 5, 8.4, 16, 18, and 28). Complicating factors included TE in 26 dogs and hypocalcemia in 42 dogs. Follow-up information was available for 445 of 469 cases (94.8%) from 23 publications, and disease-associated death occurred in 54.2%, (204 survivors and 241 dead or euthanized because of PLE). Various negative prognostic factors were reported in 6 separate studies and are listed in Table 3. Factors repeatedly identified include medium bodyweight (2 studies),[65,69] altered serum urea (3 studies),[52,56,57] and decreased serum albumin (4 studies).[52,56,57,69]

In summary, published reports to date show that PLE in dogs is associated with lymphangiectasia in around 50% of cases and LPE in approximately 66%. Lymphangitis and crypt disease each occur in <10%, and other types of IBD (granulomatous, eosinophilic) are rare causes of PLE. Immune suppressive treatment is often prescribed,

TABLE 2 Types of data presented in published papers on PLE in dogs (single case reports excluded)

First author	Study n	Deaths due to PLE (%)	CIBDAI • and CCECAI *	Clinical history	Clinical exam	Clinical pathology	Imaging	Endoscopic description	Endoscopic score	Histology description	WSAVA score	Diet	Treatment	Survival
Equilino 2015[59]	29	17 (74)	• *	-	-	•	•	-	-	•	-	•	•	•
Okanishi 2014[63]	24	3 (13.6)	*	-	-	•	-	•	-	•	•	•	•	•
Simmerson 2014[64]	30	11 (40.7)	-	•	•	•	•	-	-	•	•	•	•	•
Goodwin 2011[13]	15	10 (66.7)	*	•	•	•	-	-	-	•	-	-	-	-
Willard a 2003[65]	6	4 (66.7)	-	•	•	•	-	•	-	•	-	•	-	-
Kull 2001[12]	17	6 (35.3)	-	•	•	•	•	-	-	•	-	-	-	-
Dandrieux 2013[67]	27	15 (55.6)	-	-	-	•	-	-	-	-	•	•	•	•
Nakashima 2015[68]	92	53 (60.0)	• *	-	-	•	-	-	-	-	•	•	•	•
Kimmel 2000[50]	5	2 (40)	-	•	•	•	•	•	-	•	-	•	•	•
Littman 2000[51]	76	51 (72.9)	-	•	-	•	-	-	-	•	-	•	•	•
Van Kruiningen 1984[55]	4	2 (66.7)	-	•	•	•	-	-	-	•	-	-	-	-
Watson 2008[56]	6	1 (20.0)	-	•	-	•	•	-	-	•	•	•	•	•
Gianella 2017[57]	59	26 (44.1)	*	•	•	•	-	-	-	•	-	•	•	•
Allenspach 2017[60]	43	22 (51.2)	*	-	-	•	-	-	-	-	-	•	•	•
Rudinsky 2017[58]	11	4 (44.4)	*	•	-	•	•	-	-	•	-	•	•	•
Willard b 2003[61]	2	1 (50)	-	•	•	•	•	•	-	•	-	•	•	•
Djikstra 2010[62]	17	11 (64.7)	•	•	-	•	-	-	•	•	•	-	•	•

• indicates data present, - indicates data absent.

Abbreviations: CCECAI, canine chronic enteropathy clinical activity index; CIBDAI, canine IBD activity index; PLE, protein-losing enteropathy; WSAVA, World Small Animal Veterinary Association.
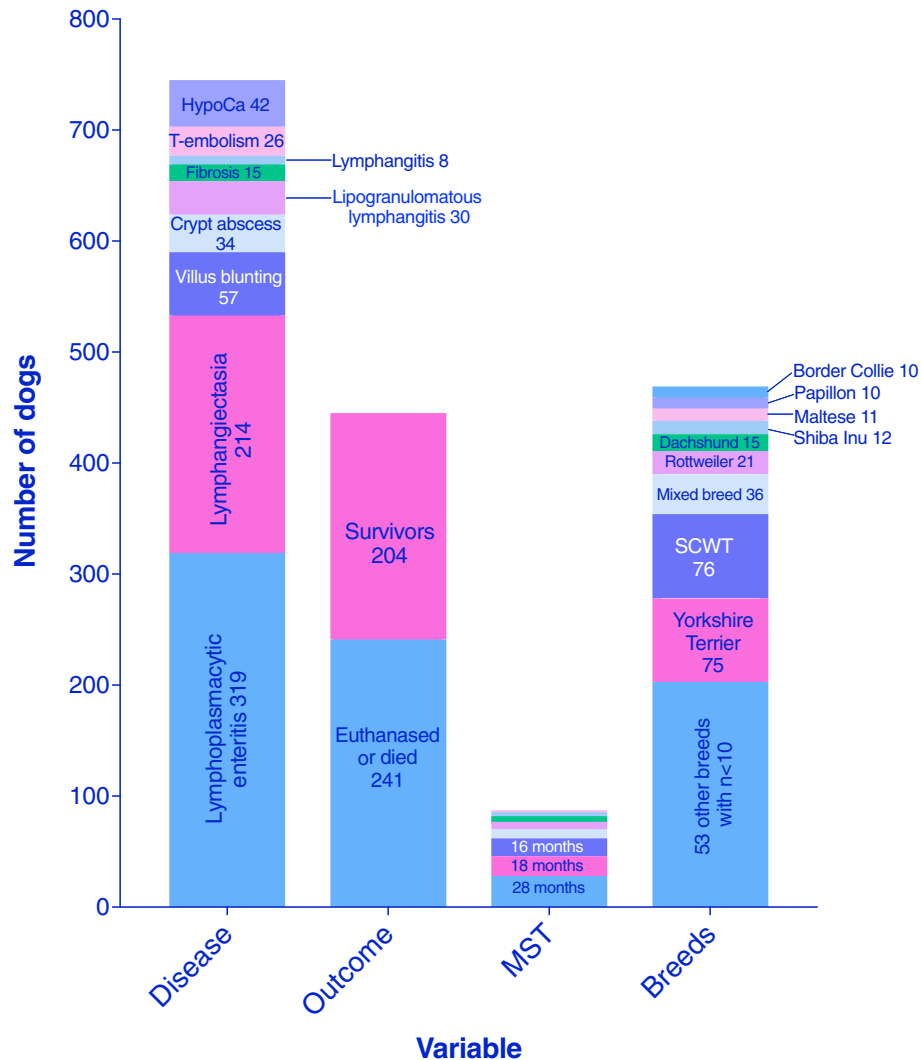
FIGURE 3 Cumulative data from 23 PLE articles spanning from 1977 to 2017, involving a total of 469 dogs. HypoCa, hypocalcemia; MST, median survival time; PLE, protein-losing enteropathy; SCWT, Soft Coated Wheaten Terrier; T-embolism, thromboembolism[12,13,49–69]

usually corticosteroids, and sometimes 2 or 3 immune suppressive agents concurrently. Dietary modification is frequently but not always implemented. Although prolonged survival can occur, over half of dogs (54.2%) with PLE die from their disease or from pulmonary thromboembolism (PTE) as a complicating factor. It is interesting to note that, in contrast, disease-associated deaths from 2 published outcome studies of IBD in dogs[71,73] were considerably lower at 13%[73] and 18%,[71] suggesting that the PLE underlying disease mechanisms are not merely a more severe continuum of IBD spectrum pathophysiology. Perhaps, this is not unexpected when considering the functions of lymph fluid in immune defense, and the role of albumin in systemic immunomodulatory and anti-inflammatory activities, antioxidant properties, and endothelial stabilization.[74]

2.8.3 | Lymphangiectasia in dogs

Primary IL, a diagnosis of exclusion, is a relatively rare problem of unknown etiology in dogs. Breed predispositions support a genetic susceptibility in the Soft Coated Wheaten Terrier (SCWT), Norwegian Lundehund, Yorkshire and Maltese Terriers, and Shar-Pei.[11] Studies of PIL with scanning electron microscopy in dogs show grossly dilated

lacteals that rupture and leak protein-rich fluid into the lumen and interstitium (Figure 1).[19] Lymph is supposedly a local tissue irritant, causing inflammation and granuloma formation,[46] which presents a unique challenge in the histological determination of primary versus secondary IL, also lymphangitis and vasculitis. Other potential diagnostic pitfalls are the similar appearance of blood and lymphatic endothelium on routine stains, and that the drainage function of lymphatics during inflammation stimulates the appearance of new vessels (lymphangiogenesis).[3,75,76] Consequently, diagnosis of IL is not straightforward, especially without the use of differentiative histological markers. The immunohistochemical markers Prox-1 and CD31 separate lymphatic from blood capillary endothelium.[75] In the SCWT, lymphatic dilatation (17% prevalence) is reported to be strongly associated with lymphangitis (35% prevalence), and less so with IBD, whereas the presence of all 3 lesions concurrently was rare.[49]

Secondary IL results from direct blockade to lymphatics, for example, neoplasia, or an indirect block, for example, dense inflammatory reaction and impaired drainage. It is reported that IL exists in a high proportion of endoscopic biopsies (44/83; 53%) from dogs with chronic GI disease, and in this patient population, there was a
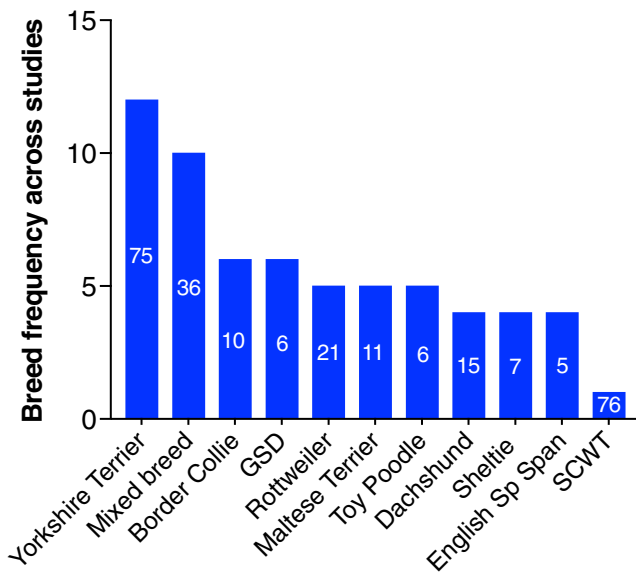
FIGURE 4 Breed frequencies represented across canine PLE publications; numbers on bars indicate the number of dogs within the breed.[12,13,49–69] PLE, protein-losing enteropathy

significant association of IL with hypoalbuminemia (28/37; 76%).[77] In close agreement, on full thickness biopsy, 59% (38/64) of dogs with chronic GI disease were shown to have IL.[78]

Published evidence showing an immune-mediated etiology of IL in dogs (and people) is totally lacking.[11,79] The obvious place to seek an anomaly is in the lymphatic endothelium or in the fine collagen fibers that anchor lymphatic capillaries to surrounding connective tissue, but none has ever been described.

2.8.4 | Granulomatous lymphangitis

Lymphangitis and granuloma formation are rare in dogs with PLE.[11] Clinical presentation is typical for PLE but can also include fever and abdominal pain.[51] The histological description is of transmural intestinal granulomas containing large "multinucleate giant cells," neutrophils,
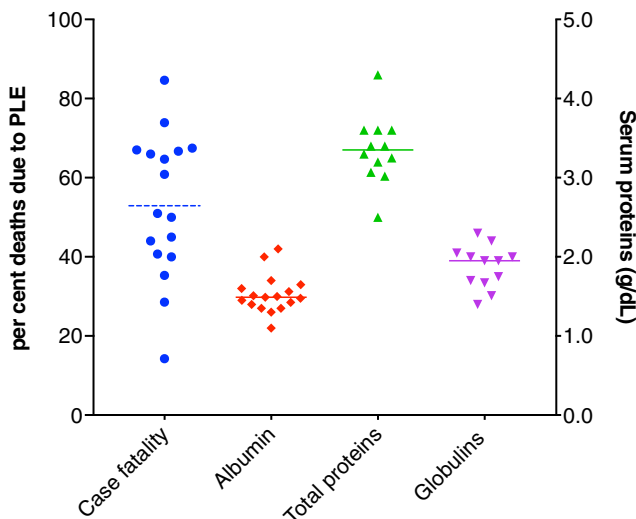

FIGURE 5 Cumulative disease-associated deaths and serum proteins reported in PLE publications (solid line median, dotted line mean).[12,13,49–57,61,64–67,69] PLE, protein-losing enteropathy
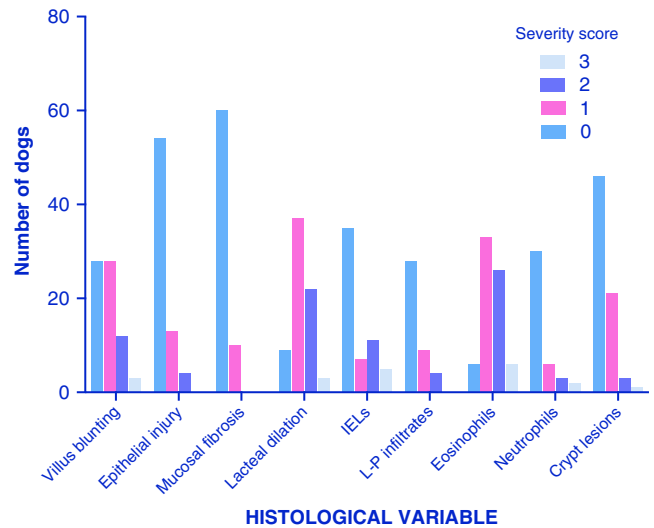

FIGURE 6 Cumulative histology scores from 3 canine studies of PLE, per the WSAVA GI standardization group scoring system.[48,53,65] GI, gastrointestinal; PLE, protein-losing enteropathy. Numbers on bars are the number of dogs

macrophages, and lymphoplasmacytic infiltrates surrounding lymphatics.[50,80] The lesions of granulomatous lymphangitis (GL) are not always localized to the bowel. In some cases involvement of the entire mesentery, LNs, and intrathoracic lymphatics goes undetected until laparatomy or necropsy because lymphatic vascular imaging and magnetic resonance imaging (MRI) are not routine procedures.[50,80] Across species, granulomatous bowel disease is particularly associated with infectious agents, for example, Whipple's Disease in people and *Tropheryma whipplei*, *Mycobacterium paratuberculosis* in Johne's disease of cattle, and *Escherichia coli* in Granulomatous Colitis of the Boxer dog.
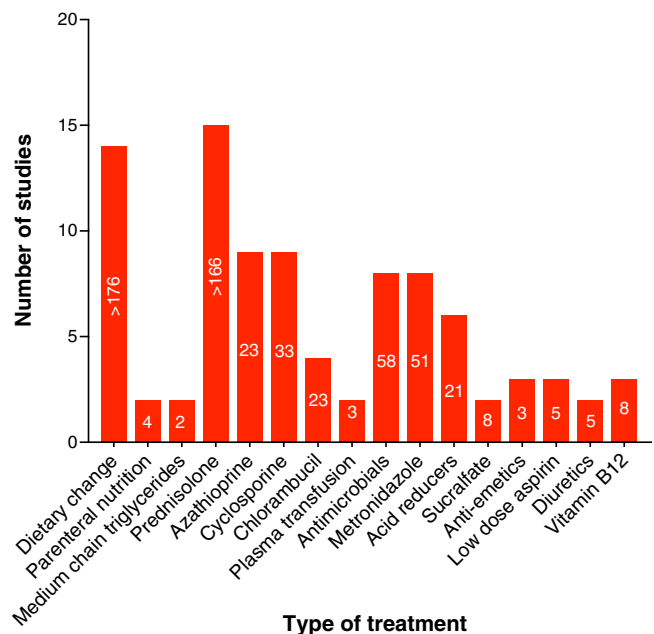

FIGURE 7 Cumulative treatment data in PLE across studies; white numbers on each bar indicate the minimum number of dogs that received each specific treatment.[49,51–57,61,64–67,69] PLE, protein-losing enteropathy

TABLE 3 Negative prognostic indicators and other findings from published reports of PLE in dogs (1977–2017)

First author	Study n	Prognostic indicators	Other findings
Equilino 2015[59]	29	NEGATIVE: • **Bodyweight** (11 to 20 kg P = 0.041). • Mildly increased serum CRP concentration (P = 0.004). • Normal serum levels of calprotectin (P = 0.031) and calgranulin (S100A12, P = 0.047).	• Serum alpha1-PI concentrations were lower in dogs with PLE (P < 0.001) vs food responsive disease (FRD). • Serum CRP (P < 0.001) was higher in dogs with PLE (median, 13.0 mg/L) vs FRD (median, 1.4 mg/L). • Moderate correlation (r = 0.659; P < 0.01) between CIBDAI and CCECAI scores.
Okanishi 2014[63]	24		Serum CRP level did not differ significantly between pre-treatment and 1 or 2 months post-treatment in responders and non-responders.
Simmerson 2014[64]	30	Significant predictors of death within 4 months of diagnosis were: 1. Vomiting (P = 0.015) 2. Monocytosis (P = 0.005) 3. Decreased serum **BUN** (P = 0.008) 4. Decreased serum **albumin** concentration (P = .027) 5. Intestinal villus blunting (P = 0.033).	
Dandrieux 2013[67]	27	• Dogs with **albumin** >14.3 g/L had a shorter duration of primary treatment than dogs with albumin <13.0 g/L (P = 0.040). • **Longer** duration of primary treatment was associated with: 1. **Bodyweight** (from 9.6 to 24.7 kg, P = 0.04). 2. Ascites presence (P = 0.013). 3. Mild lacteal dilation presence (P = 0.028).	Duration of primary treatment was shorter with chlorambucil-prednisolone treatment than azathioprine-prednisolone (P = 0.004).
Nakashima 2015[68]	92	• Responders based on normalization of CIBDAI (P < 0.0002), or CCECAI (P = 0.0004), and **albumin** (P < 0.04) showed longer survival time than non-responders. • Variables associated with shortened survival time were: 1. Higher CIBDAI score (P < 0.0003). 2. Increased serum **BUN (**P < 0.03).	• Authors include in their analysis of PLE, dogs with small-cell (19) and large cell lymphoma (10); survival data for small cell lymphoma was comparable with non-neoplastic causes of PLE. • Normalization of CIBDAI and plasma albumin concentration within 50 days of initial treatment was associated with a longer survival time.
Kimmel 2000[50]	5		Yorkshire Terriers with PLE were 9.2 times more likely to develop hypomagnesemia and hypocalcemia than other breeds.
Gianella 2017[57]	59	• Increased serum **BUN** in short-term survivors (STs, P < 0.05). • **Bodyweight** was higher in STs (P < 0.05). • Serum **albumin**, total protein and cholesterol were lower in STs (P < 0.01). • A CCECAI score of > 5 at 1 month after diagnosis was the best predictor for poor outcome.	All short-term survivors (19/59) were unresponsive to immune suppression.
Allenspach 2017[60]	43	NEGATIVE: • Treatment with immunosuppressive drugs and low serum 25(OH) D concentration at diagnosis were associated with negative outcome (p = 0.006 and p = 0.024, respectively). POSITIVE: • Higher 25(OH) D serum concentration at PLE diagnosis indicated better prognosis for survival with a hazard ratio of 0.974 for each one nmol/l increase in 25(OH) D serum concentration.	• Percentage of dogs receiving immunosuppressive drugs between outcome groups was different, with negative outcome dogs receiving more immunosuppressive drugs (p < 0.001). • A greater number of dogs treated with diet alone were in the good (versus negative) outcome group (13/22 vs 2/21, p < 0.001). • CCECAI scores between good versus negative outcome groups were not different.

In GL, an etiology is rarely found but could include infectious, parasitic, and neoplastic causes.[11,51] An immune-mediated basis is often also cited but not proven. A report of 10 dogs with GL uncovered no evidence of a bacterial cause; however unusual bacteria can evade (fluorescence in situ hybridization; FISH) detection.[80]

2.8.5 | Intestinal crypt pathology

Crypt disease is increasingly recognized as a cause of PLE in dogs although by an unknown mechanism. The YT is overrepresented in the United States and Europe for PLE,[56,64,81,82] and is particularly susceptible to crypt pathology.[56] The lesions are described as dilated cystic crypts containing sloughed epithelial cells, debris, and leucocytes[67] and

are often called "abscess" by pathologists but are not known to be associated with a specific pathogen (Figures 1 and 8). Their histological appearance, packed with proteinaceous and cellular debris, seems inconsistent, however, with simple cystic malformation. Parallels drawn with parvovirus infection, that is, villus collapse and fusion and crypt distension, led researchers to undertake intestinal immunostaining for parvovirus antigen in 2 dogs, which was negative.[67]

In people, crypt lesions are reported to be highly predictive of ulcerative colitis (UC).[83] Increased numbers of neutrophils and mucosa-associated bacteria are found to colonize crypts in UC, suggesting an opportunist role for bacteria in "cryptitis" or crypt "abscess" formation.[83,84] Investigation for a similar etiology in YT crypt PLE (YT-PLE)
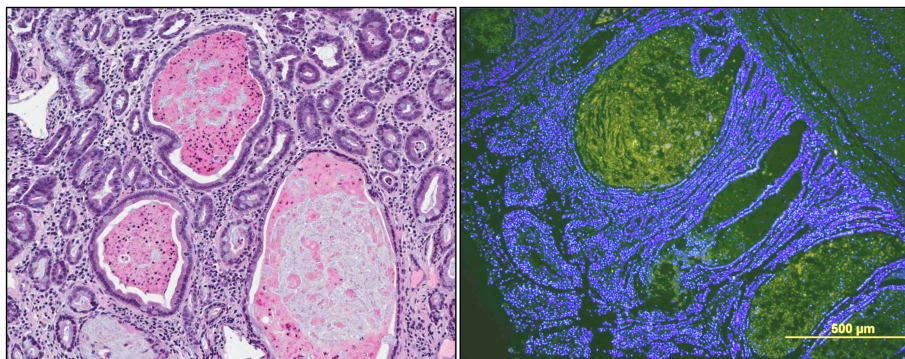
FIGURE 8 Histology of endoscopic mucosal biopsies showing small intestinal crypt lesions from a Yorkshire Terrier with PLE (H&E stain; Aperio Digital Scan, 10×, left). Florescence in situ hybridization microscopic analysis (right, magnification ×200) with eubacterial probe (red), non-eubacterial (green), and DAPI staining nuclear structures (blue) showing no evidence of intralesional or mucosally adherent bacteria.[81] PLE, protein-losing enteropathy

has been undertaken.[81] In this work, involving 12 YT (median age 8 years) presented for investigation of hypoalbuminemia (median albumin 1.6 g/dL), duodenal biopsies were evaluated (endoscopic n = 7 and surgical n = 5). Histology was dominated by large crypt lesions (median severity score 2.5/3), alongside lymphangiectasia (median score 2/3) and lymphoplasmacytic plus eosinophilic inflammation (median score 2.5/3) Analysis of intestinal biopsies using FISH with probes targeting "all bacteria" revealed no evidence of bacteria within or adjacent to crypt cysts, and the mucosal surface was infrequently colonized by bacteria (Figure 8). Most dogs were treated with immune suppressive prednisolone/dexamethasone (11/12) or azathioprine (2/12), antimicrobials (9/12), and dietary change. Within 3 months of diagnosis, 7 of 12 (58%) had died or were euthanized due to PLE (4/7) and suspected TE (3/7). Longer term survival occurred in 3 of 12 dogs, (10, 24, and 36 months) before death or euthanasia because of PLE. Two dogs were alive at 24 and 26 months after diagnosis, and 1 was still hypoalbuminemic (albumin 1.4-1.9 mg/dL). The authors concluded that crypt lesions in YT-PLE do not contain bacteria (aseptic abscess or "cyst") and found no support for localized intestinal dysbiosis in the initiation of crypt lesions. The material contained within crypts was not precisely determined but appeared to be sloughed cells and mucus/debris. The high frequency of cysts in the biopsies examined would represent a vast number if present throughout the SI, and their rupture to the lumen could plausibly cause overwhelming protein loss. A disease histologically similar to YT-PLE is "tufting enteropathy" of people, a form of epithelial dysplasia characterized by partial villus atrophy, crypt hyperplasia/pseudocyst formation, and epithelial disorganization. Mutations in the gene encoding epithelial cell adhesion molecule are associated with tufting enteropathy.[85]

2.8.6 | Lymphoplasmacytic enteritis

In dogs, IBD is currently thought to arise from immune system dysfunction, adverse reaction to dietary components, intestinal bacteria (dysbiosis), and genetic susceptibility.[86,87] In PLE, IBD ("IBD-PLE") causes protein loss either via leaky intercellular junctions or severe mucosal exudation (Figure 1). Although inflammatory processes can alone increase protein turnover, this would not usually cause hypoproteinemia unless very severe and extensive. Secondary IL can

accompany IBD, although defining primary versus secondary IL is problematic.[46,47] Now that antibodies to lymphatics are available, it might become possible to better define primary lymphatic disease versus IBD as the driving force in IBD-PLE.

There are numerous published accounts of IBD-PLE in dogs that describe the absence of lymphatic disease.[11,77,88] It is unclear whether lymphatic changes are interlinked with IBD severity; however, the idea that IBD-PLE could lack a lymphatic response deserves careful consideration when structural studies have confirmed that every villus contains a thin-walled lymphatic capillary connected to a valveless vascular network (Figures 1 and 2). The bowel is a huge organ, and in most cases the ileum and majority of the jejunum are never sampled, so that mucosal biopsies collected from approximately 10% to 15% of intestinal surface area inevitably falsely assume the full picture of pathology. Because many (>50%) dogs with PLE die caused by their disease soon after diagnosis, it becomes important to consider all possible confounding mechanisms when managing IBD-PLE. A safe assumption is a multifactorial process with varying degrees of lymphatic and intercellular losses and mucosal exudation. Targeted treatment would ideally encompass each contributing aspect, that is, cellular restoration and protection, mucosal barrier repair, resolving lymphatic obstruction, stasis and hypertension, and guarding against bacterial translocation across damaged mucosa.

3 | DIAGNOSIS OF PLE

Differential diagnosis of moderate to severe hypoalbuminemia in dogs includes hepatic failure, protein-losing nephropathy (PLN), and GI disease. In hypoalbuminemic dogs with normal preprandial and postprandial bile acids and normal urine protein : creatinine ratio, PLE is probable. Ultrasound appearance of mucosal thickening and duodenal striations/speckling are suggestive but not specific for IBD/lymphoma and lymphatic disease, respectively.[89] Presently there are no noninvasive tests for definitive diagnosis of PLE, which necessitates intestinal biopsy. The exception is lymphoma which can occasionally be diagnosed by ultrasound-guided fine needle aspiration cytology of bowel mucosa, abdominal LNs, and liver/spleen. Routine tests in PLE include serum chemistry, CBC, fecal culture and parasitology, urinalysis, abdominal and

thoracic imaging (for PTE and comorbidities), and GI endoscopy and biopsy.[11] In dogs, PLE involving severe hypoalbuminemia and effusions can occur in hypoadrenocorticism, and measuring basal serum cortisol concentration is also a consideration.[90–92]

3.1 | Noninvasive "biomarker" tests

Various potential biomarkers of IBD and PLE have been studied, such as cobalamin, C-reactive protein (CRP), perinuclear anti-neutrophil cytoplasmic antibodies, S100A12 (calgranulin-C), calprotectin, and fecal alpha-1 protease inhibitor (α_1 PI).[11,87,93–95] Cobalamin, CRP, and α_1 PI are the only commercially available tests at this time. Hypocobalaminemia is an inconsistent finding, reported in 7 of 19,[96] 8 of 13,[13] 4 of 18,[69] and 15 of 20 dogs[65] with PLE in separate studies but was associated with a negative outcome.[71] CRP was reported to be a strongly correlated marker for systemic inflammatory activity in dogs with IBD[97] but in later studies was increased in only 43% of cases[98] and did not correlate with disease severity by histologic grade or clinical scoring.[99,100] More recently it was found that a high CRP concentration is a negative prognostic indicator in PLE (P = .004),[65] but in other work, there was no difference between responders and nonresponders.[55] There is no clear consensus on the diagnostic utility of CRP, and as a nonspecific inflammatory marker, its validity across studies may depend on factors such as geographical region (endemic disease, parasitism, animal welfare standards), and laboratory methodology. More recently, fecal $\alpha 1$ PI, a measurable serum protease inhibitor lost into the bowel in PLE, was reported to distinguish dogs with moderate to severe intestinal crypt disease and lacteal dilatation (n = 54) from those without (n = 66) with sensitivity of 44%-74% and specificity of 57%-93%.[100] Being a noninvasive surveillance tool for screening PLE-susceptible breeds, α_1 PI is also helpful to detect PLE in animals with concurrent PLN or hepatic insufficiency.

Fecal calgranulin-C (S100A12) is a noninvasive marker of IBD in people.[101,102] A calcium-binding protein, S100A12, is secreted by activated neutrophils, a cell type abundant in the intestinal mucosa of IBD in people. When tested and validated for accuracy in distinguishing dogs with IBD from healthy controls, fecal S100A12 showed a sensitivity of 65% and specificity of 84%, with a misclassification rate of 20%.[100] In another study, serum S100A12 did not identify dogs with PLE; however, reference range serum S100A12 and serum calprotectin concentrations (P = .04 and P = .03, respectively), were found to be negative prognostic indicators in dogs with PLE. In people, calprotectin is another useful IBD marker,[103] corresponding to a neutrophil cytosol protein with resistance to bacterial degradation. In dogs, serum calprotectin ≥296 μg/L showed sensitivity of 82.4% and specificity 68.4% for IBD detection, although it did not correlate with CIBDAI, CRP, or histology.[104–106] The "gold standard" biomarkers for IBD and PLE in dogs have yet to be realized. The dissimilarities in histological appearance of human and canine diseases, for example, neutrophilic/granulomatous inflammation (people) versus lymphoplasmacytic inflammation (dogs and cats), and transmural (people) versus mucosal (dogs and cats) distribution, imply probable species specificity in a high performing biomarker.

3.2 | Intestinal biopsy in PLE

Definitive diagnosis of PLE necessitates histological evaluation of intestinal biopsies. Endoscopic biopsy carries lower procedural risk than surgical biopsy owing to specific comorbidities: (1) PLE is a risk for thrombosis, a "silent" killer, with risk of clot embolization or extension during anesthesia and surgical manipulations; (2) hypovitaminosis D and hypocalcemia have known potential adverse effects on wound healing, intestinal repair, cardiac and vascular function[107]; (3) hypoalbuminemia was significantly associated with death or euthanasia in 2 separate IBD outcome studies in dogs,[71,73] and 4 studies of PLE.[52,56,57,69] Logically, posing increased physiologic demands for protein (intraoperative hemorrhage and wound healing) might be reasonably expected to increase the albumin-associated risk of negative outcome. When, however, a focal intestinal mass is detected, (eg, lipogranulomatous lymphangitis, lymphoma), endoscopic reach can be a limiting factor in achieving a noninvasive definitive diagnosis, wherein surgical biopsy (with/without mass resection) might be required to facilitate an accurate diagnosis (assuming that fine needle aspirates were nondiagnostic).[50,51,80,108]

Historically, it has been queried whether endoscopic biopsy is adequate for diagnosis of IL because of failure to sample submucosal lymph vessels.[77,78,87,88,96] More recently, histological diagnosis of IL was reported as equivalent by endoscopic (44/83; 53%) and surgical biopsy (38/64; 59%).[77,78] The latter report, describing 38 dogs with IL, found transmural lymphatic pathology in 29 of 38 (76%) cases, suggesting that mucosal biopsies would usually be of adequate depth. Furthermore, the interconnected structure of the "web-like" and valveless intestinal network implies that IL would not usually be confined to submucosal lymphatics without some degree of transference of lymph capillary hypertension to the lacteal. Diagnostic accuracy of endoscopic biopsy inevitably depends on sample quality[88] and 6 (high quality) to 15 (moderate quality) duodenal samples are advised in dogs, except for diagnosis of crypt lesions, which reportedly requires 13-20 biopsies. The sensitivity of endoscopy for lymphoma diagnosis was >90%, regardless of biopsy quality.[88]

The gross appearance of the duodenal mucosa and particularly the presence of white villus tips are inadequate to accurately predict IL, with reported sensitivity of 68% and specificity of 42% for endoscopic appearance alone in IL diagnosis.[96]

The endoscopic appearance of PIL in people reveals obvious, creamy yellow villi corresponding to marked dilatation of intestinal lymphatics.[40] Histology confirms the presence of dilated mucosal and submucosal lymphatics, from a few millimeters to centimeters. Lacteals can be dilated in many villi or only a few. Mucosal abnormalities vary, but the absence of villous atrophy, significant mucosal/submucosal defect, and microorganisms, is key to diagnosis. Endoscopy can be negative when intestinal lesions are segmental or localized. In these cases, video capsule endoscopy or enteroscopy can be diagnostic.[40] Ultrasonography can show dilatation of intestinal loops, wall thickening, severe mesenteric edema, and ascites.[109] Contrast computerized tomography (CT) identifies localized IL, showing typically diffuse, nodular, small bowel thickening with or without dilatation, and a "halo sign" due to bowel swelling and edema.[42,110] Magnetic resonance imaging has been successfully applied in people with IL to evaluate for

localized and systemic disease.[111] The use of CT and MRI for the purpose of PLE diagnosis in dogs is not reported.

4 | SPECIAL TESTS

4.1 | Mucosal biopsy FISH and culture

A pathogenic role of the intestinal microbiome is not recognized in PLE; however, intestinal dysbiosis with a Gram-negative shift is emerging as a contributor to IBD pathophysiology. It should not be forgotten that the loss of immune system components in people with PLE depletes immune barrier function,[79] and so potentially, intestinal dysbiosis could be of greater severity and detriment in IBD-PLE.[83,112–115] Disrupted mucosal immunity and dysbiosis in PLE are further implied by the observation that mucosal neutrophils were significantly more often present (P = .003) in dogs with chronic enteropathy (CE) and hypoalbuminemia (17/37, 46%) than CE-normoalbuminemia (7/46, 15%).[77] In dogs at greater risk of mucosal immune compromise, for example, lymphangiectasia, ulceration, granulomatous or predominantly neutrophilic inflammation, and chronic intussusception,[116,117] analysis by FISH can be applied to search mucosae for bacterial adherence/invasion. Used in molecular microbiology to identify bacteria within formalin fixed tissues, FISH is commercially offered, at Cornell University. Where histology or FISH reveal intramucosal or mucosally adherent bacteria, intestinal specimens can be submitted for culture and sensitivity (biopsies can be stored frozen in Luria-Bertani culture medium).

4.2 | Mucosal biopsy immunophenotyping and polymerase chain reaction for antigen-receptor rearrangements

When routine histology fails to differentiate a benign, polyclonal lymphoid proliferation from a monoclonal, neoplastic process, clarification may be achieved using immunophenotyping (IP) for lymphocyte markers[118] or polymerase chain reaction (PCR) to detect clonal B and T cell populations (PCR for antigen-receptor rearrangements).[118] Both methods utilize the paraffin wax-embedded histology block, without need for further sampling. The consensus of the WSAVA International GI Standardization Group is that IP retains precedence over clonality testing as a diagnostic procedure.[72]

5 | MANAGEMENT OF PLE

In treating PLE, defining the precise nature of protein loss, that is, inflammatory, exudative, or structural (lymphatic or crypt distension) determines the selection of appropriate therapeutics. To optimize recovery rates, an otherwise safe assumption is that the processes of lymph fluid loss, active protein exudation, and leaky cell junctions could be present at some juncture in every affected bowel.

5.1 | Diet

A low-fat diet with supplementary MCT is the cornerstone of PLE management in people, proven to be the most effective, most widely

prescribed approach and with minimal adverse effects.[40,119] Desai et al. showed that within a few weeks, a high-protein, low-fat diet with MCT not only improves clinical signs but also reduces mortality in people.[37] The need for dietary control in people appears to be permanent, because clinical and biochemical findings reappear after low-fat diet withdrawal.[40] Dietary fat restriction prevents engorgement of intestinal lymphatics with chyle, preventing rupture of grossly distended lacteals. Medium-chain triglycerides are directly absorbed into the portal venous circulation and provide nutrient fat without lacteal engorgement.[40] The benefit of MCT should not be assumed to be restricted to lymphatic bypass. Medium-chain triglyceride digestion is rapid and simple, without stimulating cholecystokinin secretion or utilizing the actions of bile and lipase for absorption. Absorption of MCT occurs via passive diffusion along the GI tract into the portal system bound to albumin. No further packaging or modification of MCT molecules is required. Moreover, MCTs are not dependent on the carnitine acyltransferase system for transport into the mitochondria for β-oxidation. This provides the ability for more rapid metabolism of MCTs and improved utilization even in states of protein deficiency.[120]

Suggested dietary proportions for dogs with PLE are calories from carbohydrate 55-60%, total fat calories 10-15%, and protein calories 25-30%, using a highly digestible protein (>87%).[36] The canine diet must always contain LCFAs for provision of EFAs, and supplementation of fat-soluble vitamins (A, D, E, K) is particularly important when dietary fat content is low. Available low-fat commercial diets are listed in Table 4; however, the choice of diet in PLE cases is often dictated by patient appetite and preference. Many (especially YT) are historically fussy eaters (perhaps reflecting more chronic subclinical GI disease), and are frequently anorexic. Nutritional management in PLE is paramount and if not effectively implemented, the cycle of protein leak and catabolism will continue until eventually death is inevitable. In selective eaters or those with unreliable appetite, the authors routinely place an esophagostomy tube (E-tube) during the endoscopy anesthesia. This step seems to be the key to success in PLE management for both patient (particularly YT) and owner. Often very unwell and with critically low serum proteins, these patients need every modicum of support. The pet-owner bond can be disrupted when owners force their pet to take medications and a specific diet, and life can become unbearably stressful and anxious for both. The authors' experience is that in retrospect, the feeding tube was not infrequently a life-saving measure. When tube feeding is initiated, a commercial low fat, blenderized canned food is supplemented up to 30% protein calories (with whey or pea protein powder) and divided into 6-8 meals per day (resting energy requirement, RER, in kcal/day = 70 × [ideal weight in kg]$^{0.75}$). It should be recognized that multiple daily feedings might not be possible in every clinical scenario. If feeding is well accepted, in underweight dogs, a gradually increasing amount over the RER may be possible. In tube fed dogs, oral alimentation is encouraged, for example, lean protein (cooked turkey/fish), low fat prescription foods, and tube feeding is adjusted according to appetite. Free amino acid preparations (eg, Vivonex) can be useful during critical stages but should not replace or delay provision of complete nutritional support. To supplement caloric density, MCT can be given in the form of mixed C8 and C10 triglycerides to provide up to 30% of

TABLE 4 Range of currently available lower fat, hydrolyzed, and assisted feeding commercial diets for dogs at the time of writing

Diet	Description	Form	Protein source	Protein (%)	Fat (%)	Kcal/kg	Fiber (%)	Protein kcal (%)	Fat kcal (%)
Royal Canin GI low fat	Fat restricted	Dry	Chicken by-product meal	AF 20	AF 4.5-8.5	AF 3237	AF 1.8	24	17
				DM 24.2	DM 7.1	DM 3473	DM 3.6		
		Wet	Pork liver, pork by-products, fish oil	6.0	1.0-2.5	895	2.5	29	16
				28.8	6.5		5.2		
Royal Canin hypoallergenic	Hydrolyzed	Dry	Hydrolyzed soy protein, chicken fat, fish oil	19-21.5	10.5-17	3491	1.2	23	34
				26.2	17.5	3805	3.1		
		Wet	Peas, hydrolyzed soy, hydrolyzed chicken liver	24.5	15.1	1126	6.0	22	34
Royal Canin Anallergenic	Hydrolyzed	Dry	Hydrolyzed poultry by-products aggregate, fish oil	17	14.5	3688	4.2	17	38
				19.3	17.7	3859			
Royal Canin recovery	Assisted feeding	Wet	Chicken, chicken liver, fish oil	9.5	5.7	1112	2.6	37	59
				47.2	17.5		7.3		
Hill's i/d	Fat restricted	Wet	Pork, turkey, egg	6.7	13.9	1016	0.6	23	32
				24.6	14.8	3893	2.7		
		Dry	Chicken, chicken meal	24.5	13.7	3615	1.3	24	32
				25.7	14.8	3951	1.6		
Hill's i/d low fat	Low fat	Dry	Chicken by-product meal, pork fat	23.7	6.8	3360	1.6	25	17
				25.9	7.4	3672	1.7		
		Wet	Pork liver and by-products, turkey liver, egg whites	6.5	2.2	948	0.6	24	20
				25.1	8.5	3660	2.3		
Hill's a/d	Assisted feeding	Wet	Turkey liver, pork, chicken, egg	10.6	7.3	1173	0.4	33	55
				44.0	33.2	4796	1.3		
Hill's z/d	Hydrolyzed	Wet	Chicken liver hydrolysate	4.9	3.5	948	1.2	18	31
				20.0	14.1	3862	5.0		
		Dry	Chicken liver hydrolysate	17.6	13.3	950	4.0	17	32
				19.1	14.4	3569	4.4		
Purina EN	GI low fat	Dry	Poultry by-product meal, animal digest, fish oil	26.5	11.9	3826	1.5	26.5	28.9
				29.1	13.0		1.6		
		Wet	Liver, soy, animal by-products, turkey, salmon	10.5	4.8	1088	0.2	33.4	36.5
				39.5	17.8		0.6		
Purina EN low fat	GI lower fat	Dry	Chicken meal, animal fats	26	6.2	3550	1.1	28.3	16.4
				28.4	6.8		1.2		
		Wet	Meat by-products, chicken liver, soy protein	11.7	2.3	959	0.9	43.1	20.4
				44	8.6		3.5		
Purina HA	Hydrolyzed	Dry	Soy protein hydrolysate	19.3	9.5	3723	1.4	20.1	24.0
				21.3	10.5		1.6		

Note that different countries may have differently formulated and named products and these may also change in content over time or be renamed, (eg, Royal Canin Anallergenic in Europe is called Royal Canin Ultamino in the United States).
Abbreviations: AF, as fed (bold text); DM: dry matter (gray text); GI, gastrointestinal.

total fat calories (better achieved via an E-tube, due to variable palatability; higher levels can be ketogenic).

A measurable response should be apparent promptly if nutritional intake is appropriate in content and proportion. In severely hypoalbuminemic dogs in particular (albumin <1.5 g/dL), if serum albumin has not begun to increase after 3 days, the protein proportion can be increased to 35%, aiming to achieve positive protein balance. If there is no increase after 5-7 days, the authors switch to a different protein source, in case it should (on case-by-case basis) be more easily digested and absorbed, and better (immunogenically) tolerated. In some cases (author observation), animals achieve normalization of serum albumin but remain hypoproteinemic because of persistently low serum globulins and can respond further after supplementation with oral immunoglobulins, for example, bovine colostrum powder. Importantly, if no or only a partial diet response is initially apparent, the dog should not immediately be categorized as "food unresponsive," without further trial of other low fat, restricted antigen, or hydrolyzed diets or home-cooked food. It is feasible to begin fat restriction and dietary treatment at the animal's first presentation with GI signs and before biopsy retrieval, so that the histological picture reflects the dog's food responsiveness. This patient-centered approach can usefully inform the clinician's decisions regarding prognosis and other treatments.[53,55,64]

5.2 | Drug treatment

5.2.1 | Immune modification

Immune suppressive agents are infrequently utilized in people for the treatment of most types of PLE.[2,40–42] In contrast, in dogs an immune-mediated etiology of PLE is usually presumed[11]; however, there is no scientific or convincing volume of case-based evidence that IL (also lymphangitis and crypt disease) is autoimmune or primarily immune-mediated. Rather, the immune system can suffer the loss of components[9] such that the induction of immune suppression becomes potentially a precarious step. Lymphatic disease is a particularly unknown entity in dogs, and it is interesting to note the differing numbers of disease-associated deaths in PLE (~54%) versus IBD (<20%),[71,73] when in both diseases immune suppression with corticosteroids is the mainstay of treatment worldwide. The adverse effects of corticosteroid treatment in dogs, that is, muscle protein catabolism, thrombosis,[121–123] and hyperlipidemia[124] (with potential for lymphatic expansion) are particularly disadvantageous in PLE, and their use is somewhat counterintuitive. Recently, immunosuppressive treatment of PLE was significantly associated with a negative outcome ($P < .001$, 2/21 dogs alive), versus those treated with dietary modification alone (13/22 dogs alive).[53] Clinical response to diet alone (various foods) is also reported in in 10 of 11 YT with PLE[64] Interestingly, in a recent report[52] all "short-term" survivors (19 of 59 dogs with PLE) appeared unresponsive to immune suppressive treatment, which brings forth enquiry on how best to rationalize their use in this setting.

Immune suppression is often suggested to be reserved as "last resort" treatment, for IBD[87] and some animals with moderate to severe change are reported to clinically respond completely to dietary management alone.[71,87,125] The clinical severity of IBD does not always correlate with the histological severity score,[72] and neither variable is demonstrated to predict the requirement for immune suppressive treatment.[71,73] Commonly used immune suppressive medications in dogs are prednisolone, cyclosporine, azathioprine, chlorambucil, and mycophenolate. In a small study, improved survival of dogs with PLE was associated with the use of prednisolone plus chlorambucil (10/14 alive) versus prednisolone plus azathioprine (2/13 alive, $P = .004$).[69] Consideration of the type of inflammation present and agent pharmacodynamics should help to guide drug choice. Mycophenolate, chlorambucil, and azathioprine can all cause severe GI toxicosis and myelosuppression. Azathioprine is a purine antimetabolite that inhibits nucleic acid synthesis causing chromosomal breaks and disruption of cellular metabolism. Azathioprine is poorly absorbed PO and clinical response can take up to 6 weeks.[126,127] Chlorambucil is a cytotoxic alkylating agent with delay of up to 4 weeks before clinical response is appreciated.[126,127] It is well absorbed PO and highly bound to plasma proteins, which is of note when used in hypoproteinemic animals. Cyclosporine suppresses cell-mediated immunity and might be more efficacious in suppressing T-cell-macrophage interactions in granulomatous inflammation.[127,128] However, it is variably absorbed PO and can be less well absorbed in the diseased bowel.[129] Mycophenolate mofetil, a prodrug of mycophenolic acid, is an inhibitor of inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme in nucleotide synthesis.[130] Lymphocytes are particularly dependent on IMPDH, unlike other cell types (eg, neutrophils) that recycle nucleotides via a salvage pathway.

Mycophenolate mofetil is thought to suppress proliferation specifically of lymphocytes[130] and has many desirable properties in treating IBD: absorbed PO, "neutrophil-sparing," and without extensive time delay.[127] However, it causes significant GI irritation, and in people has a "black box" warning (serious or life-threatening risk) for lymphoma.[126] Regarding corticosteroids, budesonide is a preparation with topical activity in the GI tract but has not been specifically evaluated for PLE treatment.[127] Some practitioners use parenteral dexamethasone due to concerns that prednisolone is insufficiently PO absorbed, but in studies of people with Crohn's disease, this theory is not well supported.[131,132]

5.3 | Heparan sulfate

A common histological feature of PLE in people is loss of heparan sulfate (HS) from the basolateral surface of intestinal epithelial cells (in numerous seemingly unrelated PLE etiologies).[133] A complex proteoglycan, HS is a large, highly sulfated glycosaminoglycan composed of alternating units of α-N-acetylglucosamine and α-glucuronic acid. Heparan sulfate functions as an anticoagulant by binding ATIII and is a component of basement membranes in numerous organs, including intestinal mucosa. In the kidney, HS molecules are an important barrier against protein leak and are deficient in the glomerulus of PLN (nephrotic syndrome). Interestingly, in children born with severe PLE, deficiency of HS has been discovered in the basolateral surface of the enterocyte.[134] Exogenous heparin treatment to these children reversed enteric protein loss at doses that are considered subtherapeutic for anticoagulation in people (5000 U/day SC).[135] The precise mechanism by which HS relieves protein loss across the mucosa is unknown, but the effect has been reported in several other PLE-associated diseases.[135–137] The ability of HS treatment to reverse PLE in dogs is hopeful but has not yet been evaluated (see antithrombotics).

5.4 | Octreotide

Octreotide is a long-acting somatostatin analogue that suppresses GI motility and hormone secretion in the pituitary gland, pancreas, and intestine. In 1998, the efficacy of octreotide in 1 human PIL patient was first reported, and it has since, as monotherapy with MCT diet, been shown to lead to long-term clinical, biochemical, and histological remission of PLE of differing etiologies in people.[138] The mechanism of action of octreotide in diminishing protein loss through the GI tract is unclear. Somatostatin receptors exist in normal gut lymphoid tissue and on veins and venules.[139] Both somatostatin and octreotide normally decrease splanchnic blood flow via vasoconstriction and also decrease intestinal motility, gastric emptying, gallbladder contraction, pancreatic secretion, and triglyceride absorption.[140,141] Theorized mechanisms of octreotide's action in PIL include decreased intestinal fat absorption, inhibition of GI vasoactive peptides, and stimulation of the autonomic nervous system.[138] Octreotide is expensive and administered parenterally, and in responsive patients discontinuation usually causes PLE recurrence.[142] Reported adverse effects in human clinical trials were diarrhea, abdominal pain, nausea, headache, cholelithiasis, hyperglycemia, and constipation.[40,119,140,143] Octreotide has been used in dogs for the treatment of insulinoma.[144]

5.5 | Antithrombotics

The published data described here suggests that TE occurs in around 6% of PLE cases but is almost certainly underestimated, being a "silent" killer.[13,145,146] Prophylaxis with antithrombotic medication could prevent death and is usually well tolerated, inexpensive, and with minimal adverse effect. There is no consensus on optimal antithrombotic treatment in PLE, and options include unfractionated heparin sulfate (UFH), low-molecular-weight heparin, clopidogrel, and low-dose aspirin. In consideration of the markedly beneficial effect on PLE in people, UFH would be a logical choice but is poorly tolerated by painful SQ injection q8h. For years, UFH was thought to be minimally absorbed PO, supported by minimal changes in plasma APTT levels after oral administration, but sublingual and oral UFH absorption has since been demonstrated in numerous species including dogs.[147–149] There is now published evidence of reliable oral UFH absorption in healthy dogs, evidenced by ATIII inhibition and anti-Xa activity.[150] Recovery of UFH from both plasma and urine in people and dogs also supports that UFH administered PO is absorbed and widely distributed.[148,150] In dogs, no negative effects were observed to indicate UFH-induced hemorrhage, excessive ATIII consumption, or thrombocytopenia during oral heparin administration.[150] Further studies determining optimal oral dosage in dogs, and clinical benefit in disease states has not yet emerged. Clopidogrel and low-dose aspirin are frequently used as antiplatelet agents in dogs with PLE, either alone or in combination.[145] The optimal combination, dosages, and required duration of treatment is unknown; however, dogs in complete clinical remission of PLE can die suddenly from TE,[145] which suggests a benefit of prolonged treatment.

5.5.1 | Other

Other medications used empirically to treat IBD and PLE are antimicrobials (metronidazole, tylosin, doxycycline), gut protectants (acid reducers, sucralfate), antiemetics, and probiotics.[11,36] The use of gut protectants might help to maintain a lumenal environment that favors ongoing repair of the mucosal barrier. If mucosal ulceration or neutrophilic inflammation are present, broad spectrum antimicrobial treatment might be warranted, for example, doxycycline, amoxycillin-clavulanate, cephalexin, and FISH analysis alongside culture data would also serve to guide antimicrobial treatment. Cobalamin deficiency has been associated with a negative prognosis,[93] and supplementation by oral and parenteral routes is described.[151] Although rare, folate deficiency can be corrected by oral supplementation. Corrections of deficits in calcium and magnesium in PLE have been described by Kimmel et al.,[61] by administration of "constant rate infusions of magnesium sulfate (1.0 mEq/kg/d [0.5 mEq/lb/d], IV) and calcium gluconate solution (10 mg/kg/h, IV) until correction of the electrolyte abnormalities was achieved." Specific dosages and preparations of fat soluble vitamins A, D, E, and K in PLE are not described; however, it is unlikely that the use of a reputable veterinary supplement at the recommended daily dosage would result in vitamin toxicosis. Diagnosis of specific vitamin deficiencies and their resolution after supplementation would necessitate individual quantification of vitamin levels. Addressing concurrent problems such as nausea/emesis (eg, maropitant, ondansetron), abdominal pain (eg, tramadol, oral buprenorphine, cautious paracetamol), intestinal

dysmotility (eg, metoclopramide, cisapride) is also important. The use of appetite stimulants in animals with GI disease is controversial. In dogs, reduced appetite is nearly always an indication of another problem such as nausea, pain, dysmotility, and use of appetite stimulants can mask a debilitating untreated problem. Antioxidant treatment and reduction of environmental stressors might also be helpful.[152–155] Complications of peripheral limb edema can be avoided by using support wraps, vigilant monitoring for skin breakdown, gentle grooming and cleaning, frequent ambulation, and massage. A deep, conforming mattress will provide greater comfort when ascites and limb edema are present. Diuretics are not generally useful because the edema relates to a reduction in plasma oncotic pressure.[41]

5.5.2 | Oncotic support

Measures to increase plasma oncotic pressure such as the use of hydroxyethyl starch (Hetastarch 6%, HS), plasma transfusion, and purified canine albumin are only transiently helpful in PLE. Proteins administered IV are leaked into the GI tract, and this can in itself be detrimental due to the potential for aggravated loss of lymph and immune system components. However, this can also be life-saving in management of critically unwell patients and facilitates anesthesia and diagnostic procedures. Plasma volume expansion produced by HS approximates that of 5% albumin, for 24-36 hours. When TE is suspected, HS or purified canine albumin is preferable over plasma transfusion to avoid thrombus extension. The provision of parenteral nutrition via a central venous catheter is another means of increasing plasma oncotic pressure while providing nutritional support, with the unfortunate caveat of higher risks of sepsis, local infection/abscess, and thrombosis.[13,74,156,157]

5.6 | Theories on PLE management

5.6.1 | Stopping the leak

The lymphatic circulation is devoid of any central pump. To achieve continuous lymph flow external compression is necessary, via intestinal peristalsis, movement of body parts, arterial pulsations, and tissue compression by external forces (eg, gravitational, weight bearing, massage). When lacteals are grossly dilated, the overlapping LECs might not be able to maintain their door-like function to ensure the unidirectional flow of interstitial fluid into lymphatic capillaries (Figure 1). Without this 1-way system, the external compressive "pumps" could merely contribute to lymph entering interstitial tissues and the bowel lumen, so that an unrelenting cycle of lymph leakage, local irritation, protein loss, and edema formation develops. Anterograde lymph flow then becomes largely dependent on passive (Starling) forces. A steady state could be reached by catabolism of interstitial tissue proteins or lymphangiogenesis to restore lymph drainage, until these compensatory mechanisms are also exhausted.[158] Animals that present with panhypoproteinemia and effusions are frequently in a late stage of a dangerous "PLE cycle" disease.

It follows that diminishing the protein leak might be achieved by (1) emphasis on Starling forces (2) by modifying intestinal peristalsis, and (3) bypass of the affected intestinal area, if PLE is confirmed to be segmental.

5.6.2 | Emphasis on Starling forces

Osmolality of the human duodenum in fasted state is 200-250 mOsm/kg, versus serum osmolality, 290-300 mOsm/kg.[159] In healthy people, the SI environment changes on a time-after-food basis. The effect of different "fed states" on SI intraluminal osmolality was evaluated by administering the nutritional drink Ensure Plus.[159] Fasted state osmolality values were in general hypo-osmotic, with overall median value of 224 mOsm/kg, whereas hyperosmotic values (as high as 600 mOsm/kg, median 285 mOsm/kg) were found in fed states during the first 3 hours of digestion.[159] In dogs, fasted intestinal lumen osmolality is around 200-250 mOsm/kg and serum osmolality 290-310 mOsm/kg.[160]

Applying Starling's forces to maintain an osmotic gradient between the bowel lumen and the interstitium should be helpful in PLE management. The bowel is continually leaking across a large surface area, with no means to "plug" the leak. But by continually matching intestinal intraluminal with interstitial osmolality, an iso-osmotic oncotic gradient develops and the rate of protein loss by passive Starling forces must slow or stop. Depending on the underlying pathology (eg, lymphatic obstruction versus functional stasis), maintaining a mildly hyperosmolar lumen reverses the oncotic pressure gradient to stimulate anterograde lymph flow and protein absorption (Figure 1). It is not practicable to continually monitor lumen and serum osmolality, but an informative baseline could be measured at endoscopy. An iso- or mildly hyperosmotic lumen could theoretically be achieved by feeding small, frequent, high-protein/low-fat meals every 2-3 hours so that the bowel is in the fed state more of the time. Protein requirements in people are normally 0.6-0.8 g/kg/day, and in PLE this value may increase 5-fold (3.0-4.0 g/kg/day) before a positive protein balance is reached.[42] The optimal level of protein supplementation in dogs with PLE has never been determined. A warmed, liquid consistency, low-fat diet appears logically advantageous over dried or dense wet food to reduce GI "work" and to optimize enterocyte contact and surface tension for maximal nutrient absorption.

5.6.3 | Modifying intestinal peristalsis

Intestinal peristalsis passively facilitates lymph transport and a hypomotile bowel can develop lymph stasis. Studies of GI motility in PLE are lacking; however, bowel swelling and edema might alter GI transit times. Motility can be assessed radiographically using barium impregnated polyethylene spheres, barium fluoroscopy, radionuclide transit, or video capsule endoscopy. When dysmotility is present, prokinetic treatment, for example, metoclopramide (0.2-0.5 mg/kg PO q8h) or cisapride (0.1-0.5 mg/kg PO q8h), should be considered, with careful monitoring for signs of worsening hypoproteinemia or deteriorating clinical condition. Enhanced bowel contractility could also serve to accelerate protein and lymph loss and is likely to be more safely initiated alongside a well implemented feeding plan.

5.6.4 | Bypass of segmental PLE

Enterectomy for localized areas of lymphangiectasia has been curative in people with segmental PLE.[161] The reasons for disease localization to a particular anatomical area are unknown, but improvement can be only transient because of disease recurrence. Segmental PLE has been diagnosed using (99 m) Tc-labeled albumin scintigraphy.[162,163]

Segmental presentations in dogs and breed-specific anatomical localization are not recognized so far but are unexplored phenomena. Serial monitoring using scintigraphy (with radiolabeled canine albumin) could help to identify dogs with lesion localization that might be safely amenable to surgical treatment.

6 | CONCLUSIONS

In dogs, PLE is most frequently associated with lymphangiectasia and IBD and is managed empirically with immune suppressive agents, most frequently prednisolone, azathioprine, cyclosporine, and chlorambucil, as well as dietary change and antimicrobials. The use of immunosuppressive agents remains controversial in any of these disorders and is not utilized in people for the treatment of most types of PLE. Lifelong dietary management is the cornerstone of treatment, and recent studies in dogs support this approach.[53,64] Current treatment is associated with >50% fatality, which logically drives management toward the safest approach until pathophysiology is better understood and specific, nonempirical treatment can be formulated. The investigative history of *E. coli*-associated granulomatous colitis in the Boxer dog (GC) highlights exceptionally well that the rationale for exerting immune suppression, albeit as a "last resort" treatment, invites careful consideration. Historically, for around a half century, GC was often treated with immune suppression and with usually fatal outcome. When clinical observations with antimicrobials drove the discovery of an infectious etiology, GC became largely curable.[164,165] An excellent relationship with the internist and a high level of owner support are also a crucial aspect of managing PLE.

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