

PAPER

Prospective evaluation of a change in dietary therapy in dogs with steroid-resistant protein-losing enteropathy

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[Correction added on 17 September 2022, after first online publication: The name of the corresponding author has been corrected in this version.]

OBJECTIVE: To describe the clinical effect of dietary alteration as a sole change to therapy in dogs with steroid-resistant protein-losing enteropathy.

MATERIALS AND METHODS: Prospective study. Eligible enrolled dogs received dietary alteration as sole change to their therapeutic plan. Canine Chronic Enteropathy Clinical Activity Index and serum albumin were monitored for the 3-month study period. Long-term follow-up data were also available for some of the study participants.

RESULTS: Fifteen dogs were eligible for enrollment over the study period. Twelve were enrolled, 10 remained in the study at 30 days, nine completed the 3-month study period. Following dietary alteration, eight of 10 dogs achieved complete remission, one dog achieved partial remission and one dog had no response. Seven of eight dogs achieving complete remission have remained in remission up to 4 years following study. In dogs with complete remission, median Canine Chronic Enteropathy Clinical Activity Index score was 11.5 and 4, and median serum albumin concentration was 15 g/L and 26 g/L at 0 and 14–28 days, respectively.

CLINICAL SIGNIFICANCE: Dogs with protein-losing enteropathy with previous lack of response to a combination of dietary therapies, glucocorticoids and immunosuppressive medications can achieve remission following a dietary change. Improvement is likely to be seen within 14 to 30 days. A change in dietary approach may be an alternative to further immunosuppression or anti-inflammatory strategies in some dogs with difficult to treat protein-losing enteropathy.

Journal of Small Animal Practice (2021) **62**, 756–764
DOI: 10.1111/jsap.13334

Accepted: 04 March 2021; Published online: 13 April 2021

INTRODUCTION

Protein-losing enteropathy (PLE) is a syndrome of excessive intestinal protein loss secondary to disease affecting the enteric mucosa and

surrounding structures. While the exact mechanisms resulting in the protein loss have not been well described in veterinary medicine, it is likely the result of altered intestinal permeability, direct mucosal erosion or ulceration association with altered lymphatic function and direct loss of protein-rich lymph (Dossin & Lavoué 2011).

While potential causes of PLE are numerous (Allenspach *et al.* 2007), idiopathic inflammatory bowel disease and intestinal lymphangiectasia (IL) are considered the most common causes

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of PLE in the dog (Craven & Washabau 2019). IL is typically thought of as a congenital or primary condition, but it can also occur secondary to inflammation (Dossin & Lavoué 2011). A recent retrospective study found that 53% of dogs with chronic inflammatory enteropathies had some degree of lacteal dilation on intestinal biopsies. Of the dogs in this retrospective study who were hypoalbuminemic, 76% had lacteal dilation (Wen-nogle *et al.* 2017). In another study, 100% of dogs with lymphoplasmacytic enteritis had some degree of lacteal dilation, and lacteal villus width was inversely correlated with albumin (Rossi *et al.* 2015). In addition, lymphangiectasia can have a segmental distribution and, in some cases, be confined to deeper layers of the intestine (Larson *et al.* 2012). Therefore, it can be missed on the evaluation of routine endoscopic biopsy samples.

In humans with PLE, nutritional management is considered the mainstay of therapy. Similar importance is placed on dietary therapy in dogs with PLE (Craven & Washabau 2019). While a novel or hydrolysed protein diet is often suggested for dogs with inflammatory enteropathies, strict fat restriction is often recommended for dogs with IL. Therefore, difficulty may arise with the dietary choice when managing a patient with histopathologic evidence of both inflammatory enteropathy and lymphangiectasia, as the commercially available hydrolyzed diets generally have greater fat content than what is typically recommended for dogs with lymphangiectasia. The ideal diet for many of these dogs may be unknown at the start of therapy, and trial and error is often necessary to determine the best diet in any individual case.

In cases of refractory PLE, recommendations are often made to try alternative immunosuppressive or anti-inflammatory approaches instead of dietary interventions. Dietary interventions may not be recognised as a therapeutic target in refractory cases because studies have shown that dogs with food-responsive enteropathy tend to have normal serum albumin and lower Canine Chronic Enteropathy Clinical Activity Index (CCECAI) scores (Allenspach *et al.* 2007, Allenspach *et al.* 2016, Dandrieux *et al.* 2016). However, dietary therapy is becoming increasingly recognised as crucial component of the management of dogs with PLE, given some dogs with PLE can be successfully treated with diet alone (Rudinsky *et al.* 2017, Nagata *et al.* 2020). Furthermore, dietary fat restriction was demonstrated to be efficacious in dogs with IL refractory to prednisolone (Okanishi *et al.* 2014). However, this was a retrospective study and this phenomenon was only evaluated in dogs with IL.

The purpose of this prospective study was to assess the effect of a dietary intervention as the single change in therapy in dogs with steroid-refractory PLE. The success of the dietary change was assessed by monitoring CCECAI scores (Allenspach *et al.* 2007), and serum albumin over the course of the study. We hypothesised that many dogs with steroid-resistant PLE would be clinically responsive to a change in diet.

MATERIALS AND METHODS

Case selection criteria

Dogs presenting to the Colorado State University Veterinary Teaching Hospital with a complaint of persistent clinical signs

of gastrointestinal disease (CCECAI ≥ 5) despite at least 1 month of therapy for previously diagnosed PLE were considered eligible for inclusion in the study. The original diagnosis of PLE was based on serum albumin < 25 g/L and exclusion of other causes of hypoalbuminaemia including renal loss of protein based on either negative urine dipstick or urine protein:creatinine ratio < 0.5 , and liver disease based on normal pre- and postprandial serum bile acids or normal synthetic liver function and enzyme activity on routine biochemistry panel in all dogs. Most dogs also had hypoadrenocorticism excluded as the cause of their hypoalbuminaemia and chronic gastrointestinal signs. All dogs were screened for helminths (faecal floatation). Additionally, all dogs had serum trypsin-like immunoreactivity (TLI) concentrations measured for exclusion of exocrine pancreatic insufficiency and abdominal ultrasound performed before endoscopy. All dogs had endoscopic exam and gastrointestinal histopathology. Only dogs with primary findings of inflammatory enteritis or IL were eligible for the study. Dogs were not eligible for the study if their primary diagnosis was intestinal neoplasia. Dogs were excluded from the study if significant concurrent disease was present (*e.g.* extra-intestinal disease, neoplasia).

Dogs were eligible for inclusion if they had been treated with prednisone or prednisolone at a dosage of at least 1 mg/kg, PO, q24h, or divided q12h for at least 30 days with persistence of CCECAI score ≥ 5 . A total of at least 30 consecutive days of therapy with prednisone or prednisolone within the previous 3 months was required for inclusion. If dogs were currently receiving prednisone or prednisolone at the time of enrollment, it was preferred that the dose not be adjusted for the first 30 days of the study, unless significant adverse effects were occurring. Following 30 days, tapering of steroid was allowed. No dose increases were permitted at any time during the study.

In order to be eligible for the study, no other major changes to the dogs' current therapies outside of the dietary adjustment were allowed at the time of enrollment. This included changes to immunosuppressive therapy, or the addition of new therapies such as cobalamin or calcitriol. If the dogs were receiving immunosuppressive therapy (*e.g.* azathioprine, chlorambucil, cyclosporine) at the time of enrollment they were eligible for inclusion only if that therapy had been administered for > 30 days at appropriate doses and with the provision that dose could not be altered for the first 30 days of the study. Tapering of these drugs was allowed following the initial 30-day period. Dose increases were not allowed.

The type of dietary adjustment was determined by the primary attending clinician and a board-certified veterinary nutritionist (JS) and was partly based on the avoidance of specific and general types of diets that were previously attempted but determined to be ineffective, or were diets that the dog had not accepted. Diets chosen were at the discretion of the primary attending clinician and veterinary nutritionist, however, Royal Canin® diets including Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Moderate Calorie, and Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF were available at no cost to the owner

for the duration of the initial study period (30 days). Each dog underwent a one-week diet transition period where the new diet was gradually introduced in increasing proportion towards a complete transition. Dogs had to accept the diet and owners agreed to feed the diet exclusively.

Monitoring and determination of clinical response

Dogs were re-evaluated at 14 to 30 days and 3 months to determine clinical response by use of CCECAI score and biochemical parameters. A partial response (PR) was defined as improvement of clinical signs but persistence of CCECAI score >3 . A complete response (CR) was defined as resolution of clinical signs as evidenced by CCECAI score decreasing to insignificant levels (0 to 3) (Allenspach *et al.* 2007) within the study period. Non-response was defined by failure to improve CCECAI score. The decision to intervene with significant treatment changes in the first 30 days of the study would result in removal from the study. Owners provided informed consent and agreed to recheck evaluations for their dogs at 14 to 30 days, and 3 months following study enrollment. The Institutional Animal Care and Use Committee at Colorado State University approved this project.

Statistical analysis

Descriptive statistics were calculated for age, bodyweight, sex and clinical variables.

RESULTS

Twenty-three dogs with refractory PLE were assessed for eligibility over the study period. A flow diagram detailing case enrollment, removal and completion is presented in Fig 1. Fifteen dogs with steroid-refractory PLE were eligible for enrollment in the study over the time period. In three cases, the owners could not commit to feeding the diet exclusively. Therefore, 12 dogs were enrolled. Two dogs did not eat the recommended diet exclusively following the allowed one-week transition period; therefore 10 dogs remained in the study. Nine dogs completed the 3 month study; one dog was euthanased at 30 days due to PLE.

Seven dogs were neutered males, two dogs were spayed females and one dog was an entire female. Median age was 5 years (range, 1 to 8 years). Median bodyweight was 24 kg (range 2.6 to 46 kg). Median body condition score (BCS) (0 to 9 scale) (Laflamme 1997) was 4 (range 2 to 5). Breeds included mixed breed (three), Yorkshire terrier (three), Bernese mountain dog (two), Australian shepherd (one) and Labrador retriever (one). The diagnosis of PLE had been made in all dogs via intestinal biopsy and exclusion of other possible causes of hypoalbuminaemia. All dogs had significant proteinuria excluded with a negative dipstick ($n=8$), or with a urine protein:creatinine ratio <0.5 ($n=2$). Three dogs had fasted and post-prandial bile acids performed, two of three were within normal limits for both (fasted

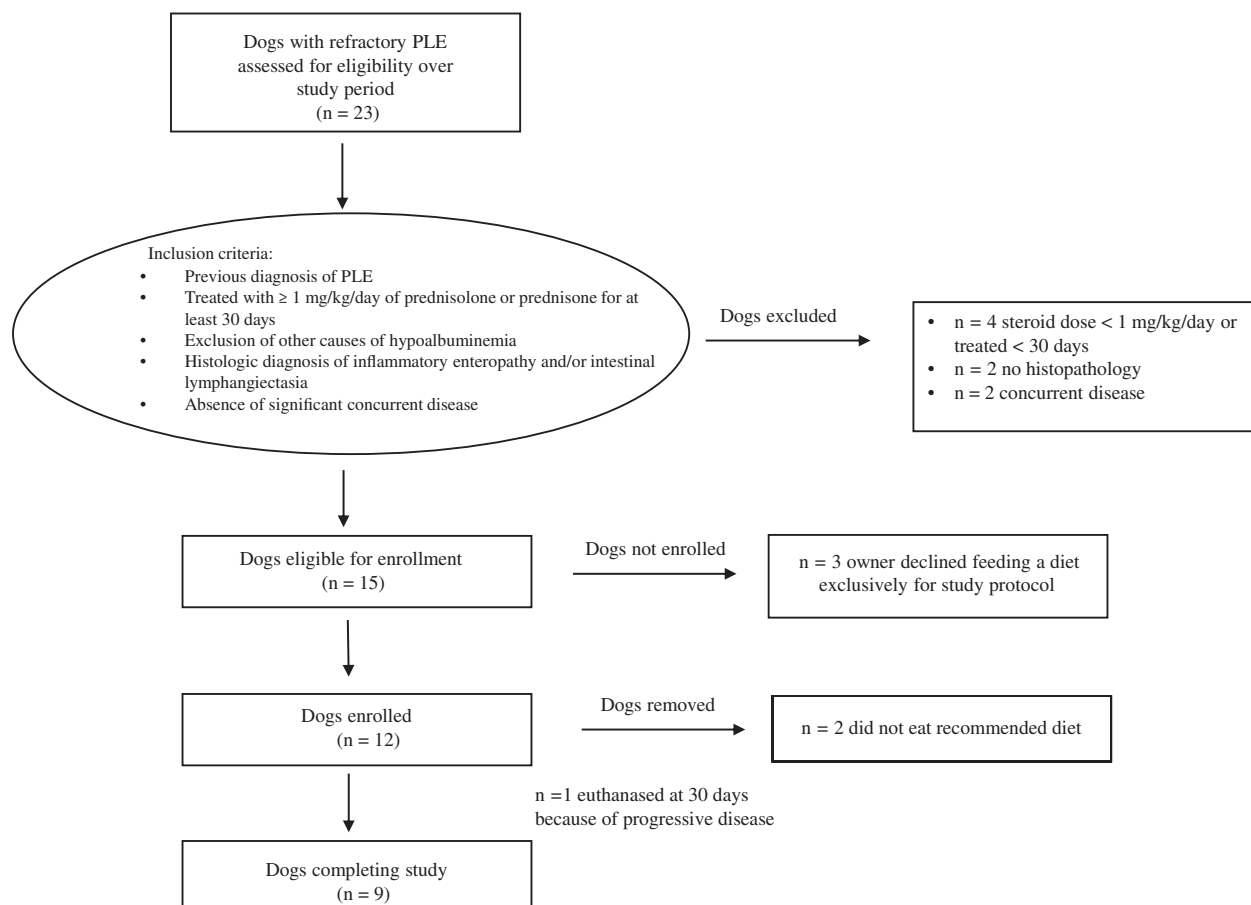


FIG 1. Flow diagram detailing case enrollment, removal and completion

<5 µmol/L; post prandial <25 µmol/L) and one dog had mild elevations of both fasted and post prandial bile acids (fasted 56 µmol/L; post-prandial 41 µmol/L). The dog with mildly elevated bile acids was panhypoproteinaemic with no other significant suggestion of liver dysfunction. Three dogs had only fasted bile acids performed all the within normal reference interval (<5 µmol/L). In the other four dogs, significant liver disease was considered very unlikely to be the cause of hypoalbuminaemia based on the presence of normal synthetic liver function and enzyme activity on routine biochemistry panel ± the presence of panhypoproteinaemia (three of four dogs). Eight of 10 dogs had a basal cortisol >55.18 nmol/L or normal adrenocorticotropin hormone (ACTH) stimulation test to exclude hypoadrenocorticism as the cause of hypoalbuminaemia. The other two dogs were administered glucocorticoid therapy before testing for hypoadrenocorticism and remained on glucocorticoid therapy at the time of enrollment so an ACTH stimulation test could not reliably be performed. All dogs had exocrine pancreatic insufficiency excluded by fasted serum canine TLI >5.0 ng/mL. Additionally, all dogs had an appropriate history of faecal testing with or without deworming, and all dogs had abdominal ultrasound by a board-certified radiologist to screen for intestinal or abdominal masses before endoscopic biopsy.

Five dogs had gastroduodenoscopy with endoscopic biopsy of the stomach and duodenum. Five dogs had gastroduodenoscopy and ileocolonoscopy with biopsy of the stomach, duodenum, ileum and colon. Only the small intestinal results are reported here. Duodenal cellular infiltrate was described as lymphoplasmacytic in six dogs, lymphoplasmacytic with eosinophils in two dogs, lymphoplasmacytic with neutrophils in one dog, and lymphoplasmacytic with eosinophils and neutrophils in one dog. The degree of duodenal cellular infiltrate was classified as mild in five dogs, moderate in four dogs and severe in one dog (lymphoplasmacytic with neutrophils) based on WSAVA criteria (Washabau *et al.* 2010). Ileal cellular infiltrate was lymphoplasmacytic in two dogs, lymphoplasmacytic with eosinophils in two dogs, and lymphoplasmacytic with eosinophils and neutrophils in one dog. The degree of ileal cellular infiltrate was described as mild in all five dogs. The ileal cellular infiltrate type differed from the duodenal cellular infiltrate type in one of five dogs. The degree of ileal cellular infiltrate differed from the degree of duodenal cellular infiltrate in two of five dogs. All dogs had WSAVA scoring for morphologic changes of the duodenum performed. Lacteal dilation was absent in six dogs and noted as mild in four dogs. Crypt distension was mild in three dogs, and marked in one dog. Further descriptive data on the 10 study dogs from the time of original diagnosis is shown in Tables 1 and 3.

At the time of enrollment in the study, all 10 dogs were exhibiting diarrhoea. Other complaints included lethargy (n=6), weight loss (n=7) and vomiting (n=1).

At enrollment, eight of 10 dogs were receiving prednisone or prednisolone with a median dose of 0.86 mg/kg, PO, q24h or divided q12h (range 0.5 to 1.5 mg/kg/day). The median duration of therapy was 1.75 months (range 1 to 3 months). All dogs had received prednisone or prednisolone as an initial therapy at the time of diagnosis. The median starting dose was 1.5 mg/kg,

Table 1. Selected clinicopathologic data at time of original diagnosis

Laboratory value	Median (range)	Reference interval
Albumin	16 (13 to 22)	30 to 43 g/L
Globulin	15.5 (13 to 35)	15 to 32 g/L
Cholesterol	2.5 (2.1 to 4.5)	3.4 to 7.8 mmol/L
Total calcium	2 (0.97 to 2.5)	2.3 to 2.9 mmol/L
Ionised calcium	1.19 (0.7 to 1.4) [†]	1.25 to 1.45 mmol/L
Magnesium	0.66 (0.45 to 0.78)	0.74 to 0.99 mmol/L
Lymphocyte	1.3 (0.5 to 3.2)	1 to 4.8×10 ⁹ /L
Platelet	344 (176 to 636)	200 to 500×10 ⁹ /L
Cobalamin	258 (<150 to 794)	251 to 908 ng/L
Folate	10.5 (3.5 to 20)	7.7 to 24.4 mcg/L

[†]Data available for seven dogs

PO, q24h or divided q12h (range 1.0 to 2.0 mg/kg/day) and all dogs received this dosage for a minimum of 1 month.

One dog not receiving prednisolone or prednisone at the time of enrollment had been weaned off prednisone 1 week prior, following 4 months of therapy with an initial dosage of 1.5 mg/kg, PO, q24h for 1 month and subsequent tapering, with no noted clinical or clinicopathologic improvement. Additionally, this dog developed a splenic thrombosis diagnosed via abdominal ultrasound, which influenced the decision to wean off prednisone therapy in this case. The second dog not receiving prednisolone or prednisone at the time of study entry had been weaned off prednisolone 2 months prior, following 2 months of therapy with an initial dosage of 1.1 mg/kg, PO, q24h for 5 weeks and subsequent tapering following limited clinical or clinicopathologic improvement and the perception of significant adverse effects (polyuria, polydipsia, polyphagia and behavioural changes) reported by the owner.

At the time of enrollment, five dogs (including one of the dogs not receiving prednisolone or prednisone therapy) were receiving immunosuppressive medications for >30 days including chlorambucil (n=1; 5 mg/m², PO, q24h), azathioprine (n=1, 2.2 mg/kg, PO, q 48 hours), leflunomide (n=1, 1 mg/kg, PO, q24h) and cyclosporine (n=2, 5.5 to 8.1 mg/kg, PO, q24h). Two additional dogs had been treated with cyclosporine (6 to 10 mg/kg, PO, q24h) but were no longer receiving it. One other dog had been treated with leflunomide (2 mg/kg, PO, q24h) but was no longer receiving it.

Other medications that were being utilised at the time of enrollment included aspirin (n=3, 0.5 to 1.1 mg/kg, PO, q24h), calcium carbonate (n=1, 20 mg/kg, PO, q12h), clopidogrel (n=3, 2 mg/kg, PO, q24h), cobalamin (n=9, 250 to 1000 mcg, SQ, q weekly), calcitriol (n=2, 5 to 10 ng/kg, PO, q12h), famotidine (n=1, 0.6 mg/kg, PO, q12h), furosemide (n=1, 1 mg/kg, PO, q12h), magnesium sulphate (n=2, 1 to 2 meq/kg, PO, q24h), maropitant (n=1, 1.6 mg/kg, PO, q24h), metronidazole (n=1, 12.8 mg/kg, PO, q12h), Provia® (seven strain probiotic) (n=2, 1 capsule, PO, q24h), tylosin (n=4, 15 to 25 mg/kg, PO, q12h) and sucralfate (n=1; 1 g, PO as slurry, q 8 h).

Diets utilised in the study included Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF Dry and Canned (n=4), Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Moderate Calorie Dry and Canned (n=1), and a veterinary nutritionist-formulated home cooked diet (n=5). Prescription diets that

Table 2. Composition of the diets fed at the time of enrollment and the diets used in the study

Diet	Protein source	% Fat [†]	% Pro [†]	% CHO [†]
Hill's® Prescription Diet® z/d® Canine Dry	Chicken hydrolysate	32	17	51
Purina® Pro Plan® Veterinary Diet HA Hydrolyzed Canine Formula (Vegetarian)	Soy protein isolate hydrolysate	24	20	56
Royal Canin Veterinary Diet® Canine Hydrolyzed Protein Adult HP Dry	Soy protein isolate hydrolysate	43	19	38
Royal Canin Veterinary Diet® Canine Hydrolyzed Protein Adult HP Canned	Soy protein isolate hydrolysate	34	22	44
Royal Canin Veterinary Diet® Canine Ultimino®	Chicken feathers hydrolysate	38	17	45
Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Moderate Calorie Dry	Whitefish	25	26	49
Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Canned	Whitefish	41	25	34
Royal Canin Veterinary Diet® Canine Gastrointestinal Low Fat LF Dry	Chicken	17	24	59
Royal Canin Veterinary Diet® Canine Gastrointestinal Low Fat LF Canned	Chicken, pork	16	29	55
Tilapia/quinoa [‡]	Tilapia	15	44	40
Turkey/cous cous [‡]	Turkey	12	39	49
Tuna/potato [‡]	Tuna	10	53	37

[†]% metabolizable energy (ME) Pro protein, CHO carbohydrate (numbers shown are from the formulations available during the study time period, some diets have been reformulated since)

[‡]Full recipe not included so not complete and balanced as described, should not be fed exclusively for extended period of time without appropriate supplements

Table 3. Response, histopathology, dietary details and outcome for the 10 dogs in the study

Response	Biopsy duodenum (inflammatory infiltrate [^] /degree; LD score)	Diet at time of enrollment/diet fed exclusively? (yes/no/unknown)	Study diet	Outcome/last known status
CR1	LP/Moderate; Mild	Hills z/d#/yes	Royal Canin GI LF*	Clinical remission at 30 months Albumin (30 months): 31 g/L Tx: Royal Canin GI LF*, B12, psyllium
CR2	LP/ Mild; None	Purina HA@/yes	Royal Canin GI LF*	Clinical remission at 28 months Albumin (24 months): 39 g/L Tx: Royal Canin GI LF*, B12
CR3	LPN/Severe; None	Purina HA@/yes	Royal Canin PW%	Clinical remission at 12 months Albumin (12 months): 28 g/L Tx: Royal Canin PW%, prednisone 0.25 mg/kg/day, B12, tylosin
CR4	LPE/Moderate; None	Royal Canin HP&/no	Tuna/potato\$	Relapse at 12 months, euthanized at 15 months
CR5	LPEN/Mild; Mild	Royal Canin PW%/yes	Tilapia/quinoa\$	Clinical remission at 12 months Albumin (12 months): 31 g/L Tx: turkey/oat\$, budesonide 0.025 mg/kg/day, B12
CR6	LPE/Mild; Mild	Royal Canin GI LF*/yes	Tuna/potato\$	Clinical remission at 48 months Albumin (48 months): 41 g/L Tx: tuna/potato\$, prednisone 0.125 mg/kg/day, B12
CR7	LP/Mild; Mild	Royal Canin Ultimino~/yes	Turkey/cous cous\$	Clinical remission at 12 months Albumin (12 months): 34 g/L Tx: turkey/cous cous\$, B12, calcitriol
CR8	LP/Mild; None	Royal Canin GI LF*/unknown	Tilapia/quinoa\$	Clinical remission at 12 months Albumin (12 months): 32 g/L Tx: tilapia/quinoa\$, prednisone 0.25 mg/kg q 48h, aspirin
PR1	LP/Moderate; None	Royal Canin HP&/yes	Royal Canin GI LF*	PR at 3 months, relapse and euthanasia at 6 months Albumin (3 and 6 months): 24 g/L & 19 g/L
NR1	LP/ Mild; None	Royal Canin PW%/yes	Royal Canin GI LF*	No response at 30 days (euthanasia) Necropsy: endocardial thrombosis

CR Complete response, PR Partial response, NR No response, MC Male castrated, FS Female spayed, FI Female intact, LD Lactate dilation, [^]LP Lymphoplasmacytic, Tx Treatments at the time of last known status, LPE Lymphoplasmacytic with eosinophils, LPN Lymphoplasmacytic with neutrophils, LPEN Lymphoplasmacytic with eosinophils and neutrophils, #Hill's® Prescription Diet® z/d® Canine Dry, *Royal Canin® Canine Gastrointestinal Low Fat™ Dry and Loaf Canned, @Purina® Pro Plan® Veterinary Diet HA Hydrolyzed Canine Formula (Vegetarian), % Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Moderate Calorie Dry and Loaf Canned, &Royal Canin Veterinary Diet® Canine Hydrolyzed Protein Adult HP Dry and Loaf Canned, Royal Canin Veterinary Diet® Canine Ultimino®, \$full recipe not included so not complete and balanced as described, should not be fed exclusively for extended period of time without appropriate supplements

had been attempted at various times before enrollment in the study included Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF (n=4), Royal Canin Veterinary Diet® Canine Selected Protein Adult PW Moderate Calorie (n=2), Royal Canin Veterinary Diet® Canine Hydrolyzed Protein Adult HP (n=7), Purina® Pro Plan® Veterinary Diet HA Hydrolyzed® (n=3), Hills Prescription Diet® i/d® Low Fat (n=1), Royal Canin Veterinary Diet® Ultimino® (n=1), Royal Canin Veterinary Diet® Canine Selected Protein Adult PV (n=1) and Hills Prescription Diet® z/d® Canine

(n=2). Nutritional information for the study diets and the diets being fed at the time of enrollment is shown in Table 2. The decision for a board-certified veterinary nutritionist to formulate a customised home-cooked diet was based on expected patient acceptability of the diet or previous non-response to both a veterinary prescription hydrolyzed diet and veterinary prescription low-fat diet. Of the eight dogs that were classified as having CR, two were treated with Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF, one was treated with Royal Canin Veterinary

Diet® Canine Selected Protein Adult PW Moderate Calorie and the other five were treated with board-certified veterinary nutritionist home-cooked diets supplemented with vitamins and minerals (Table 2). Two dogs were treated with the tuna/potato diet, two dogs were treated with the turkey/cous cous diet, and one dog was treated with the tilapia/quinoa diet. The dog treated with the tilapia/quinoa diet was transitioned to turkey/oat 3 months after completion of the study due to palatability issues and return of some clinical signs. Improvement was noted on the turkey/oat diet, which remains his current diet at the time of publication. The dog who had a PR and the dog with NR were treated with Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF. Data for the 10 dogs in the study including response, histopathology, dietary details and outcome are presented in Table 3.

At enrollment, median CCECAI score was 11.5 (range 6 to 14) and median albumin was 15 g/L (range 15 to 25 g/L). At 2 to 4 weeks median CCECAI score was 4 (range 1 to 11), and median serum albumin was 26 g/L (range 18 to 31 g/L). Data is available for all dogs at 3 months except for the dog who had no response and was euthanased at 30 days. At 3 months, median CCECAI score was 1, and median serum albumin was 28 g/L (range 19 to 30 g/L). Data is shown for the nine dogs with a complete data set in Figs 2 and 3. CCECAI scores and serum albumin concentra-

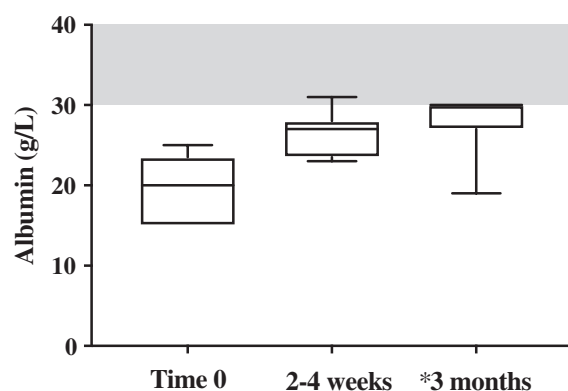


FIG 2. Change in serum albumin over time period of study. *Data available for nine of 10 dogs. Whiskers depict minimum and maximum values. Horizontal bar represents median. Lower and upper quartiles also shown. Institutional reference interval depicted by the grey bar

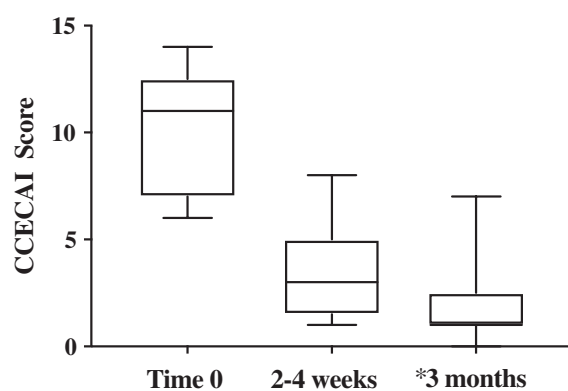


FIG 3. Change in CCECAI score over time period of study. *Data available for nine of 10 dogs. Whiskers depict minimum and maximum values. Horizontal bar represents median. Lower and upper quartiles also shown

tion were available for all dogs at all time points in the study with the exception of the dog who was euthanased at 30 days. Three of the eight dogs with a CR were last evaluated at 12 to 30 months post-study enrollment at which time their serum albumin ranged from 28 to 39 g/L. They were lost to follow up after that time. Four of the CR dogs remain in clinical remission at the time of publication 12 to 48 months post study enrollment. The most recent serum albumin concentration in those dogs ranged from 31 to 41 g/L. The final CR dog experienced relapse at 12 months following a change to his therapeutic protocol (addition of other ingredients to home-cooked diet and adjustment of immunosuppressive regimen) and was euthanased. The dog with PR was euthanased at 6 months post study enrollment. The dog with NR was euthanased 30 days after study enrollment; necropsy was performed and revealed endocardial thrombosis and mild lymphoplasmacytic enteritis with mild lacteal dilation.

DISCUSSION

This study evaluated the clinical effect of a dietary change in 10 dogs with steroid-resistant PLE. Eight dogs achieved a CR with medium to long-term survival reported in seven of those dogs. Of those seven dogs, three responded to a veterinary prescription diet and four responded to a veterinary nutritionist-formulated home-cooked diet.

The exact reason for the response to dietary change in these cases is unknown. In seven of nine dogs that achieved a complete or PR during the study period, the dietary therapy recommended and implemented was more fat-restricted than the diets the dogs had previously been fed. In the cases of the veterinary nutritionist-formulated home cooked diets, the dietary fat restriction was also beyond what is found in commercially available diets (Table 2). Notably, the tilapia/quinoa diet is only slightly lower in fat than the commercially available low-fat diets. The study dog fed the tilapia/quinoa diet was previously eating Royal Canin Veterinary Diet® Gastrointestinal Low Fat LF Dry and Canned, thus fat restriction was not likely to be a major factor in that case, however, it is not clear if she was eating her previous diet exclusively. Dietary fat restriction has previously been shown to be effective in allowing for the reduction in prednisone dosage in 24 dogs with IL and PLE (Okanishi *et al.* 2014). In addition to IL, intestinal inflammatory infiltrates were noted in 18 of 24 dogs in that study (Okanishi *et al.* 2014). While lymphatic dilation based on WSAVA scoring was generally absent to minimal in our study dogs, the presence or extent of lymphatic disease may have been underappreciated. The diagnosis of lymphangiectasia can be hindered by the fact that the disease can be segmental, and in some cases confined to the deeper parts of the intestinal wall (Larson *et al.* 2012). A recent study used immunohistochemistry (IHC) to label the intestinal lymphatic vasculature in endoscopic biopsies from dogs with chronic inflammatory enteropathies. This study found that some dogs had lymphangiectasia in the proprial mucosa, but not in the villous lacteals. This lymphangiectasia was not identified on routine haematoxylin and eosin evaluation (Wennogle *et al.* 2019). Furthermore, of the nine dogs

that responded partially or completely, four did not have ileal or jejunal biopsies obtained. It is possible that more significant lacteal dilation would have been observed on ileal (Casamian-Sorrosal *et al.* 2010, Procoli *et al.* 2013), jejunal or full-thickness surgical biopsies. If concurrent lymphatic disease was under-recognised in this group of dogs with PLE, dietary fat restriction may have played a significant role in the positive responses seen. Alternatively, fat restriction may benefit dogs with PLE even in the absence of documented or suspected IL as dietary fat restriction could lead to improvement in secretory or osmotic diarrhoea in dogs with impaired fat digestion (Rudinsky *et al.* 2018). While we cannot conclude this from our study, it is possible that dietary fat restriction beyond what is found in commercially available diets may be necessary in some cases of PLE and may possibly have resulted in the clinical response seen in some of the dogs fed a veterinary nutritionist-formulated home cooked diet.

The clinical improvement of some dogs in this study could also be attributed to providing a novel protein source compared to the diet the dogs were eating previously, and a subsequent reduction in some component of an adverse food reaction (Gaschen & Merchant 2011). However, of the dogs achieving a CR, only two of eight were switched from a non-novel to novel protein source. Importantly, however, both the PR and NR dogs in the study were switched from a novel to a non-novel protein source in an attempt to provide more significant fat restriction compared to the diets they were previously fed. It is unknown whether those dogs would have responded to a non-novel diet with more significant fat restriction (ultra-low-fat diet) or would have benefitted from a diet that was both novel in its protein source and low in fat. Controlled dietary trials investigating specific dietary approaches in patients with PLE are needed to help clinicians determine what aspect of dietary management (fat restriction, providing a hydrolyzed or novel protein source, optimising digestibility or some combination of these approaches) are important in individual patients with PLE.

Five dogs in this study were treated with a veterinary nutritionist-formulated home-cooked diet. All dogs responded well initially; one dog experienced a relapse at 12 months following changes to his therapy and was subsequently euthanased. Although no direct conclusions can be formed from this study, there are several reasons why a veterinary nutritionist-formulated home-cooked diet may be beneficial in dogs with PLE. First, a home-cooked diet can provide both a novel protein source and achieve dietary fat restriction beyond what is found in commercial diets. When utilising a veterinary nutritionist to formulate a homemade diet, the individual diet can be formulated with the intention to identify a novel protein in each case, and to adjust the fat content as dictated by a complete dietary history. While some dogs with inflammatory enteropathies and secondary lymphangiectasia will respond to therapies addressing just the inflammation, it is possible that in some cases dogs need management of both conditions. Finally, veterinary nutritionist-formulated home cooked diets typically contain few ingredients, so it could also be considered that the absence of certain ingredients, rather than the dietary protein source or fat content, could benefit some patients with PLE (Simpson & Jergens 2011).

Finally, it is possible that improved digestibility of the diet may have played a role in the clinical improvement seen in some dogs in this study, however this is difficult to assess in individual client-owned dogs.

Dietary trials are often considered a preliminary treatment in dogs with chronic enteropathies based on evidence that up to 66% of dogs will be responsive to intervention with food alone (Rudinsky *et al.* 2018). However, there is a tendency in clinical practice to consider dogs to be non-responsive to food after a single trial with one type of diet, and often the validity of these trials cannot be determined. In one study of 23 dogs with chronic enteropathies responding to a hydrolyzed or easily digestible diet, nine had been previously determined to be non-responsive to food (Mandigers *et al.* 2010). Furthermore, the tendency to distinguish dogs as either food-responsive, steroid/immunosuppressive-responsive, or non-responsive may undermine the complexity of the disease process in cases of chronic enteropathies with or without concurrent PLE. A recent study found that dogs with food-responsive enteropathy had significantly lower CCECAI scores than dogs with immunosuppressive-responsive or non-responsive PLE. The authors of this study determined an optimal cut-off CCECAI of 8 for determining food-responsive from immunosuppressive-responsive or non-responsive PLE (Nagata *et al.* 2020). The dogs in our study had a median CCECAI score of 11.5 at the time of enrollment. Although only a small number of dogs, the results of our study would suggest that even in dogs with severe disease, it may be just as important to optimise dietary therapy as it is to adjust anti-inflammatory or immunosuppressive therapy in some cases of PLE.

In dogs that achieved CR, an improvement in CCECAI score and serum albumin was observed between time of enrollment and at the first recheck, 14 to 30 days after enrollment. Although this is just a small number of dogs, this suggests that clinical and biochemical improvement that may be attributed to a dietary change in dogs with PLE would likely be seen within 14 to 30 days of the dietary change, which is in line with previous studies (Marks *et al.* 2002, Allenspach *et al.* 2007, Allenspach *et al.* 2016).

Following the change in diet, three dogs have maintained control of their disease beyond the study period without glucocorticoids or other immunosuppressives for 12 to 36 months (last known or time of publication). The other four surviving dogs have maintained remission with a combination of therapies including diet, low doses of glucocorticoids (<0.5 mg/kg/d, PO, q24h) and supportive therapies. The ability to manage PLE dogs without the use of glucocorticoids or with low doses of glucocorticoids is desirable, as steroids can have significant side effects (Cohn 2010) and can negatively affect quality of life. Furthermore, a hypercoagulable state has been identified in dogs with PLE (Goodwin *et al.* 2011), which could be worsened with the use of glucocorticoids. Additionally, the use of long-term immunosuppressive drugs can increase the risk of opportunistic infections, lymphoproliferative disorders, and other adverse effects (Viviano 2013). Dietary modifications have limited side effects and known efficacy for the treatment of gastrointestinal disease. Prospective clinical trials are needed in a variety of dogs with PLE

to determine whether certain specific dietary approaches (e.g. hydrolyzed, low fat or veterinary nutritionist-formulated home cooked diet) with or without the use of concurrent anti-inflammatory drugs and/or immunosuppressives are best, perhaps in relation to clinical, clinicopathologic or histologic characteristics.

This study has limitations. First, this study is significantly limited by the small number of dogs included. The enrollment of dogs with significant intestinal disease in a prospective study requiring adherence to a strict diet and limiting the changes to therapy that are allowed made case recruitment challenging. Dogs were enrolled in the study after the diagnosis of PLE had been established which limited some aspects of the study. Gross endoscopic scores could not be obtained for all dogs. Ileal biopsies were not obtained in all dogs. Pathology can differ between sections of intestine therefore this may have limited the understanding of the disease process in those cases (Casamian-Sorrosal *et al.* 2010, Procoli *et al.* 2013). Additionally, even with the use of the WSAVA guidelines for histopathologic scoring (Washabau *et al.* 2010) of endoscopic intestinal biopsy samples, substantial interobserver variability occurs (Willard *et al.* 2002), and scoring is still considered subjective (Jergens *et al.* 2014). Ideally, a single pathologist blinded to the case data would have evaluated all of these samples; however, samples were not available for further review in all cases. The ability to draw firm conclusions from this study is also limited by the variability in fat reduction and protein source when compared to the previous diet. Future studies are needed in which these dietary factors are carefully controlled.

It is impossible to be certain whether the remission achieved in these cases was due to the dietary change alone because many dogs were receiving other treatments as outlined above. Furthermore, a control group was not included so spontaneous improvement or response to other treatments cannot be entirely ruled out. Before enrollment, all dogs had received prednisone or prednisolone at 1 to 2 mg/kg/day for a time period of at least 30 days, but at enrollment in the study various doses of steroids were being utilised in addition to other medications. Four dogs that responded were receiving immunosuppressive therapies that may also have influenced their response. Neither drug levels nor suppression of T-cell function were measured for dogs on immunosuppressive therapies, so efficacy of those drugs was not determined. However, all dogs had been receiving their immunosuppressive drugs for >30 days before enrollment and three of four dogs were able to be weaned off these drugs following their diet change, suggesting that these drugs may not have been the most important component of their therapy. Many dogs were also treated with supportive therapies before enrollment and continued on those medications after enrollment. The contribution of those therapies cannot be determined. Nine dogs were being supplemented with cobalamin; however, levels were not re-assessed in all cases so it is unknown if hypcobalaminaemia may have contributed to inadequate or lack of response in some dogs. In eight of 10 dogs, a basal cortisol or ACTH stimulation test was performed and excluded hypoadrenocorticism as the cause of clinical signs and hypoalbuminaemia. Hypoadrenocorticism could not be completely excluded in the other two dogs due to current glucocorticoid therapy. However, previous

lack of response to glucocorticoid therapy would suggest that hypoadrenocorticism was unlikely in these cases. The dog with NR, a Yorkshire terrier, had mild-moderately elevated fasting and post-prandial serum bile acids; however, this dog was pan-hypoproteinemic suggesting that intestinal disease was at least a component of his hypoalbuminaemia. Based on his synthetic liver function tests and liver enzyme activities liver failure was considered unlikely in his case. Considerations for the cause of the dog's elevated serum bile acids include portal vein hypoplasia, common in Yorkshire Terriers (Hirose *et al.* 2014), or ileal disease (Lawrence & Steiner 2017). Four dogs had hepatic failure excluded on the basis of synthetic liver function tests and liver enzyme activities alone as fasting and post-prandial serum bile acids were not performed. However, all of these dogs responded to the dietary change, which would not be expected in cases of liver failure.

PLE has a variable but guarded prognosis (Nakashima *et al.* 2015), and euthanasia is common after repeated failure of multiple therapies. If clients are willing and able, consultation with a board-certified veterinary nutritionist and new dietary approaches should be considered in dogs refractory to standard treatments. Prospective clinical trials are needed to evaluate the optimal dietary therapy and to determine if dietary therapy alone or in combination with other therapies is best for individual dogs with PLE.

Acknowledgements

The authors would like to acknowledge the support of Royal Canin® as well as the Naniboujou Research Legacy Fund in completion of this project. Drs. Wennogle and Webb have received honoraria for speaking engagements from Royal Canin®. Royal Canin® contributed funding for this study.

Conflict of interest

Drs. Wennogle and Webb have received honoraria for speaking engagements from Royal Canin®. Royal Canin® contributed funding for this study.

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