**Clinicopathologic and prognostic factors in short- and long-term surviving dogs with protein-losing enteropathy**

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## Introduction

Protein-losing enteropathy (PLE) in dogs results from severe small intestinal disease that allows leakage of protein into the intestinal lumen (Dossin and Lavoue, 2011). While panhypoproteinemia associated with loss of albumin and globulin is the most common clinicopathological abnormality, isolated albumin loss can also be observed (Willard et al., 2000; Allenspach et al., 2007). The major causes of PLE in dogs are intestinal lymphangiectasia, inflammatory bowel disease, and lymphoma (Craven et al., 2004; Dandrieux et al., 2013; Nakashima et al., 2015). Because PLE is associated with decreased serum albumin and increased loss of $\alpha_1$-PI into the gastrointestinal tract, measurement of serum albumin and fecal $\alpha_1$-proteinase inhibitor ($\alpha_1$-PI) should be included in the diagnostic workup (Murphy, 2003; Willard, 2013). But since the $\alpha_1$-PI test is not readily available, PLE is usually diagnosed after excluding other conditions associated with hypoalbuminemia and intestinal histopathology (Dossin and Lavoue, 2011; Willard, 2013). As compared with chronic enteropathy (CE) with normal albumin (Craven et al., 2004; Allenspach et al., 2007; Simpson and Jergens, 2011), the prognosis for PLE is usually considered guarded (Allenspach et al., 2007; Dossin and Lavoue, 2011), and the response to therapy is variable (Simmerson et al., 2014).

Moreover, information on factors that predict outcome of PLE at diagnosis is limited and long-term follow-up data are lacking. Negative prognostic indicators include medium size (11 to 20 kg), high canine IBD activity index (CIBDAI) score, a history of vomiting, monocytosis, mildly increased C-reactive protein, normal serum calprotectin and S100A12 concentrations, clonal rearrangement of lymphocyte antigen receptor genes, and intestinal villous blunting (Simmerson et al., 2014, Equilino et al., 2015; Nakashima et al., 2015). Information on the impact of serum albumin and blood urea nitrogen concentrations on outcome or survival time is controversial. One study found the survival time to be significantly influenced by low blood urea nitrogen concentration and severity of hypoalbuminemia (Simmerson et al., 2014), two others reported that elevated blood urea nitrogen concentration and hypoalbuminemia, but not its severity, were negatively correlated with outcome (Owens et al., 2011; Nakashima et al., 2015), and another found that outcome or survival time were not significantly influenced by the initial serum albumin concentration (Equilino et al., 2015).

Finally, while many dogs with PLE secondary to CE die shortly after initiation of treatment, there are some that achieve prolonged survival.

The aim of this study was to retrospectively evaluate the differences in clinical and clinicopathological findings between short- and long-term surviving dogs with PLE secondary to CE at diagnosis and after treatment, and to identify potential risk factors for poor outcome.

## Animals, Material and Methods

*History and laboratory findings*

We retrospectively reviewed the medical records of dogs with PLE secondary to CE diagnosed at three different hospitals between January 2009 and November 2013. Inclusion criteria were complete history and physical examination findings, chronic gastrointestinal signs lasting for more than 3 weeks, hypoalbuminemia (< 2 g/dL) of gastrointestinal origin with or without hypoglobulinemia, and histopathological evidence of gastrointestinal inflammation on biopsies collected by endoscopy or laparotomy. Histologic examination was performed in all dogs according to the histopathological standards of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group. All biopsies were retrospectively reviewed by a pathologist blinded to the diagnosis. Chronic canine enteropathy clinical activity index (CCECAI) scores (Allenspach et al., 2007), complete blood count, serum biochemistry and coagulation profiles, pancreas specific lipase levels, serum folate and cobalamin concentrations were gleaned from the medical records. The reference ranges of the hospital laboratories were substantially similar. During review of the medical records (February 2015), follow-up information was obtained by telephone from the owners or referring veterinarians.

*Classification and therapy*

Dogs were classified as either short-term (STs) or long-term (LTs) survivors if they had died within or were still alive at 6 months after diagnosis, respectively. Additionally, the medical records were searched for information about the categorization of CE as food-, antibiotic-, or immunosuppressive-responsive. Dogs that showed complete remission of

clinical signs while on elimination diet (hydrolysed or restricted antigen diets) were categorized as having food-responsive CE. Dogs that showed complete remission of clinical signs while on tylosin (15 mg/kg, PO, q 12 h) or metronidazole (10 mg/kg, PO, q 12 h) were categorized as having antibiotic-responsive CE. Dogs that responded to oral prednisone (1 mg/kg, twice a day for 2-3 weeks before considering dose reduction), oral azathioprine (1 or 2 mg/kg, once a day), oral chlorambucil (4-6 mg/m$^2$, once a day for at least 2 weeks before considering dose reduction), or oral cyclosporine (5 mg/kg, once a day) were categorized as having immunosuppressive-responsive CE. Dogs were classified as immunosuppressive-unresponsive if they showed poor or no clinical response to immunosuppressives (partial disappearance or persistence of clinical signs).

Since the medical records also reported the results of repeated exams at follow-up visits , we set T1 (1 month after initiation of immunosuppressives) as the time point at which the clinical and clinicopathological information was complete for the majority of the dogs.

*Statistical analysis*

Statistical analysis was performed with a commercially available statistical data analysis program (MedCalc®). Assessment of data for normality was calculated using the D'Agostino-Pearson test. Continuous variables were expressed as mean (± sd), median (minimum and maximum), percentages or both. Categorical variables were expressed as normal/negative (0) or abnormal/positive (1). Fisher's exact test was used to compare between the STs and the LTs the variables: sex, complaints/clinical signs (small bowel diarrhea, mixed diarrhea, decreased appetite, increased appetite, vomiting, peripheral edema, ascites, pleural effusion, pruritus, polyuria and polydipsia, lethargy, and muscular twitching/convulsions), results of the SNAP cPL® test, coagulation profile at T0, and treatments with different types of immunosuppressives.

Student's t-test was used to compare between the STs and the LTs the variables: age, body weight, CCECAI scores, serum albumin, folate concentrations, and lipase activity at T0 and the CCECAI scores, serum albumin and globulin concentrations at T1. The Mann-Whitney test was used to compare between the STs and the LTs the variables: number

of monocytes and platelets, globulin, serum total protein, total cholesterol, blood urea nitrogen, magnesium, cobalamin and fibrinogen concentrations at T0 and the serum total protein, magnesium and total cholesterol concentration at T1. Values of $P < 0.05$ were considered significant. A receiver operating characteristic (ROC) curve was used to select the optimum cut-off value of the variables at T1 to discriminate the STs from the LTs.

## Results

*History, physical examination, and CCECAI scores*

We reviewed the medical records of 59 dogs with PLE secondary to CE diagnosed between January 1, 2009 and November 30, 2013. Of these 59 dogs, 19 were classified as STs and 40 as LTs. Among the STs were dogs from 9 different breeds and 2 mixed-breed dogs. Fourteen were male and 5 female. The age range was from 9 months to 13.4 years (mean, 5.9 ± 3.3), and the weight range was from 14 to 40 kg (mean, 23.6 ± 7.3). Table 1 reports the presenting complaints/clinical signs. The median duration of clinical signs prior to diagnosis was 2 months (range 1-36). The median survival time was 90 days (range 31 to 180). Among the LTs were dogs from 20 different breeds and 9 mixed-breed dogs. Twenty-two dogs were male and 18 female. The age range was from 1 to 11.6 years (mean, 6.5 ± 2.5), and the weight range was from 1.9 to 45 kg (mean 17.4 ± 12.4). Table 1 reports the presenting complaints/clinical signs. The median duration of clinical signs prior to diagnosis was 2 months (range 1-36). The median survival time was 880 days (range 210 to 1,787). No statistically significant differences in sex and age between the two groups were found at T0; body weight was significantly higher in the STs ($P < 0.05$). There was no difference in presenting complaints/clinical signs between the STs and the LTs.

CCECAI scores were available for all dogs at T0, and for all dogs except 1 at T1. No significant differences in the CCECAI scores between the groups were found at T0; at T1 the CCECAI score was significantly higher in the STs (Figure 1).

*Clinicopathological findings*

Tables 2 and 3 present the clinicopathological findings and the number of dogs that had undergone testing, respectively. At T0, no statistically significant differences between the two groups were found for: number of monocytes and platelets, serum albumin, globulin, total protein, total cholesterol, magnesium, cobalamin, folate and fibrinogen concentrations, lipase activity, results of the SNAP cPL® test, and coagulation profile. Blood urea nitrogen concentrations were significantly higher in the STs ($P < 0.05$). At T1, albumin, serum total protein and total cholesterol concentrations were significantly lower in the STs ($P < 0.01$).

*Gastrointestinal histopathology results*

Gastroduodenoscopy was performed in 58 dogs. Additional ileoscopy and colonoscopy were performed in 14 and 28 dogs, respectively. Laparotomy was performed in 1 dog. Tissue quality was classified as adequate in all cases. Moderate to marked histopathologic abnormalities in the small intestine were found in all dogs. Lymphocytic-plasmacytic inflammation (50 dogs) and lymphangiectasia (28 dogs) were the most common abnormalities. Moderate to severe lymphocytic-plasmacytic colonic inflammation was found in 24 dogs.

*Treatment and outcome*

No significant differences in the treatments with different types of immunosuppressive therapies were found between the two groups. Based on their response, all STs were categorized as immunosuppressive-unresponsive. Among the LTs, 32 dogs were categorized as immunosuppressive, 1 and 1 each as food- and antibiotic-responsive CE, respectively; 6 dogs were categorized as immunosuppressive-unresponsive.

Follow-up information was available for all dogs. Thirty-three dogs (55.9%; 31 with immunosuppressive-responsive CE; 1 with food-responsive CE; 1 with antibiotic-responsive CE) were alive at the time of medical record review (73 months), and 26 (44.1%; 19/19 STs and 7/40 LTs with immunosuppressive-responsive CE) had died because of PLE-related complications. The main cause of death was deterioration of clinical conditions presumably due to malabsorption. A cut-off CCECAI score of > 5 at T1 was found to be the best predictor for poor outcome (Figure 2).

## Discussion

With this retrospective multicenter study we compared the clinical and clinicopathological findings of 59 short- and long-term surviving dogs with PLE secondary to CE, and investigated potential prognostic factors. Consistent with previous observations, the adult dogs of any size were affected by PLE (Allenspach et al., 2007; Lecoindre et al., 2010; Dossin and Lavoue, 2011; Dandrieux et al., 2013; Simmerson et al., 2014), with a predominance of males, however (Kull et al., 2001; Simmerson et al., 2014). Medium size (11 to 20 kg) has recently been reported as a negative prognostic indicator (Equilino et al., 2015). When we compared the two groups, we observed that body weight was significantly higher among the STs. This might simply reflect the type of study population or suggest that large breed dogs might be affected by more severe forms of PLE.

Small bowel diarrhea and decreased appetite were the most common historical complaints in both groups. A recent retrospective study found that vomiting was a negative prognostic factor (Simmerson et al., 2014), however, we noted no significant differences in the presenting complaints/clinical signs between the two groups at T0. As seen also in our sample, ascites or pleural effusion are common complaints or physical examination findings in dogs with PLE (Allenspach et al., 2007, Lecoindre et al., 2010) but they do not seem to be negative prognostic indicators (Simmerson et al., 2014). Activity indices for assessing disease severity can also be used as prognostic markers (Jergens et al., 2003; Allenspach et al., 2007). According to one study, CCECAI ≥12 at diagnosis predicted refractoriness to treatment and euthanasia within 3 years (Allenspach et al., 2007). To the contrary, in our and in a recent study (Equilino et al., 2015), outcome or survival time were not significantly influenced by activity indices at diagnosis.

The only significant difference in pathologic variables between the two groups at T0 was the blood urea nitrogen concentration, which can be influenced by dehydration, renal failure or severe GI protein loss. But because we had no information about prerenal and renal azotemia values in these PLE dogs, this result should be interpreted with caution. Furthermore, the retrospective design of the present study is an additional limitation. Several variables tested at T0 were not available at T1 for all dogs, and treatments were not strictly standardized. That said, collectively, our results

may support the hypothesis that the severity of clinical signs and the majority of serum biochemistry and coagulation profile findings at diagnosis do not appear to correlate with outcome.

The prognosis for dogs with PLE in the current veterinary literature is guarded (Allenspach et al., 2007; Dossin and Lavoue, 2011). Except for a recent retrospective study (Simmerson et al., 2014), there are few reports of survival data for dogs with PLE (Craven et al., 2009; Simmerson et al., 2009; Dijkstra et al., 2010; Goodwin et al., 2011; Owens et al., 2011; Equilino et al., 2015). Although 32.2% of the dogs had died within 6 months of diagnosis, a greater proportion (55.9%) was still alive at the time of manuscript preparation, suggesting that not only PLE-affected Yorkshire Terriers, but also other PLE-affected breeds may experience remission of clinical signs and prolonged survival despite severity of clinicopathologic findings at diagnosis, as recently described (Equilino et al., 2015).

To our knowledge, no long-term data on the follow-up of dogs with PLE exist. At T1 (6 months follow-up) the CCECAI scores were higher and the albumin, total protein, and total cholesterol concentrations all lower in the STs. Moreover, the dogs with a CCECAI score > 5 were more likely to die within 6 months of initial diagnosis. Since these variables at T0 did not significantly influence the outcome, they might simply reflect a poor response to therapy. Finally, since all STs were categorized as immunosuppressive-unresponsive, it is reasonable to assume that a poor response to therapy is a poor prognostic indicator. Indeed, survival time was shorter in the dogs with high CCECAI scores at T1 and that were unresponsive to therapy.

In conclusion, the clinical outcomes of PLE are variable, with the majority of the dogs having prolonged survival despite the severity of clinicopathological findings at diagnosis.

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Table 1. List of presenting complaints/clinical signs at diagnosis (T0) in short-term (ST) and long-term (LT) survivors.

| Variables | STs<br>n/t | LTs<br>n/t |
|---|---|---|
| Small bowel diarrhea | 19/19 | 39/40 |
| Mixed diarrhea | 5/19 | 14/40 |
| Decreased appetite | 13/19 | 22/40 |
| Increased appetite | 1/19 | 3/40 |
| Vomiting | 9/19 | 24/40 |
| Peripheral edema | 1/19 | 5/40 |
| Ascites | 6/19 | 20/40 |
| Pleural effusion | - | 3/40 |
| Pruritus | 1/19 | 4/40 |
| Polyuria and polydipsia | 4/19 | 5/40 |
| Lethargy | 2/19 | 8/40 |
| Muscular twitching/convulsion | 1/19 | 4/40 |

n= number of dogs showing the complaint/clinical sign

t= total number of dogs

Table 2. Summary of laboratory results at diagnosis (T0) in short-term (ST) and long-term (LT) survivors

| | STs | | LTs | | |
|---|---|---|---|---|---|
| Variables | Positive or abnormal | Mean (±SD) | Positive or abnormal | Mean (±SD) | Reference values |
| Albumin | - | 1.49 (±0.42) g/dL | - | 1.45 (±0.31) g/dL | 2.80-3.70 g/dL |
| Globulin | - | 2.08 (±0.87) g/dL | - | 1.88 (±0.61) g/dL | 2.80-4.20 g/dL |
| Total Protein | - | 3.57 (±0.97) g/dL | - | 3.32 (±0.75) g/dL | 5.60-7.90 g/dL |
| Total Cholesterol | - | 118 (±54) mg/dL | - | 122 (±48) mg/dL | 140-350 mg/dL |
| Magnesium | - | 1.67 (±0.86) mg/dL | - | 1.88 (±1.90) mg/dL | 1.60-3.20 mg/dL |
| Blood Urea Nitrogen | - | 37.62 (±18.65) mg/dL | - | 28.72 (±17.32) mg/dL | 18-55 mg/dL |
| Cobalamin | - | 219 (±128) ng/L | - | 229 (±107) ng/L | 250-730 ng/L |
| Folate | - | 9.95 (±5.80) µg/L | - | 10.42 (±5.90) µg/L | 7-17 µg/L |
| Fibrinogen | - | 450 (±146) mg/dL | - | 555 (±225) mg/dL | 150-450 mg/dL |
| Lipase | - | 191 (±123) U/L | - | 302 (±205) U/L | 70-700 U/L |
| SNAP cPL® | 2 | - | 3 | - | - |
| Number of platelets | - | 463,684 (±218,761) cells/µL | - | 426,056 (±252,977) cells/µL | 150,000-500,000 cells/µL |
| Number of monocytes | - | 686 (±482) cells/µL | - | 634 (±396) cells/µL | 100-1400 cells/µL |
| Coagulation profile | 9 | - | 10 | - | - |

257 Table 3. Summary of clinicopathological variables tested at diagnosis (T0) and 1 month after initiation of immunosuppressive therapy
258 (T1) in short- term (ST) and long-term (LT) survivors.

259

| | T0 | | T1 | |
|---|---|---|---|---|
| Variable | STs n/t | LTs n/t | STs n/t | LTs n/t |
| Albumin | 19/19 | 40/40 | 18/19 | 40/40 |
| Globulin | 19/19 | 40/40 | 18/19 | 39/40 |
| Total Protein | 19/19 | 40/40 | 18/19 | 39/40 |
| Total Cholesterol | 19/19 | 38/40 | 13/19 | 31/40 |
| Magnesium | 9/19 | 26/40 | 6/19 | 18/40 |
| Blood Urea Nitrogen | 19/19 | 37/40 | - | - |
| Cobalamin | 13/19 | 30/40 | - | - |
| Folate | 13/19 | 32/40 | - | - |
| Fibrinogen | 5/19 | 16/40 | - | - |
| Lipase | 10/19 | 27/40 | - | - |
| SNAP cPL® | 5/19 | 17/40 | - | - |
| Number of platelets | 19/19 | 38/40 | - | - |
| Number of monocytes | 18/19 | 32/40 | - | - |
| Coagulation profile | 6/19 | 13/40 | - | - |

260 n=number of dogs in which the variable was measured

261 t=total number of dogs

262

Figure 1. Comparison of canine chronic enteropathy activity scoring index (CCECAI) between short-term (ST) and long-term (LT) survivors at T0 and T1.

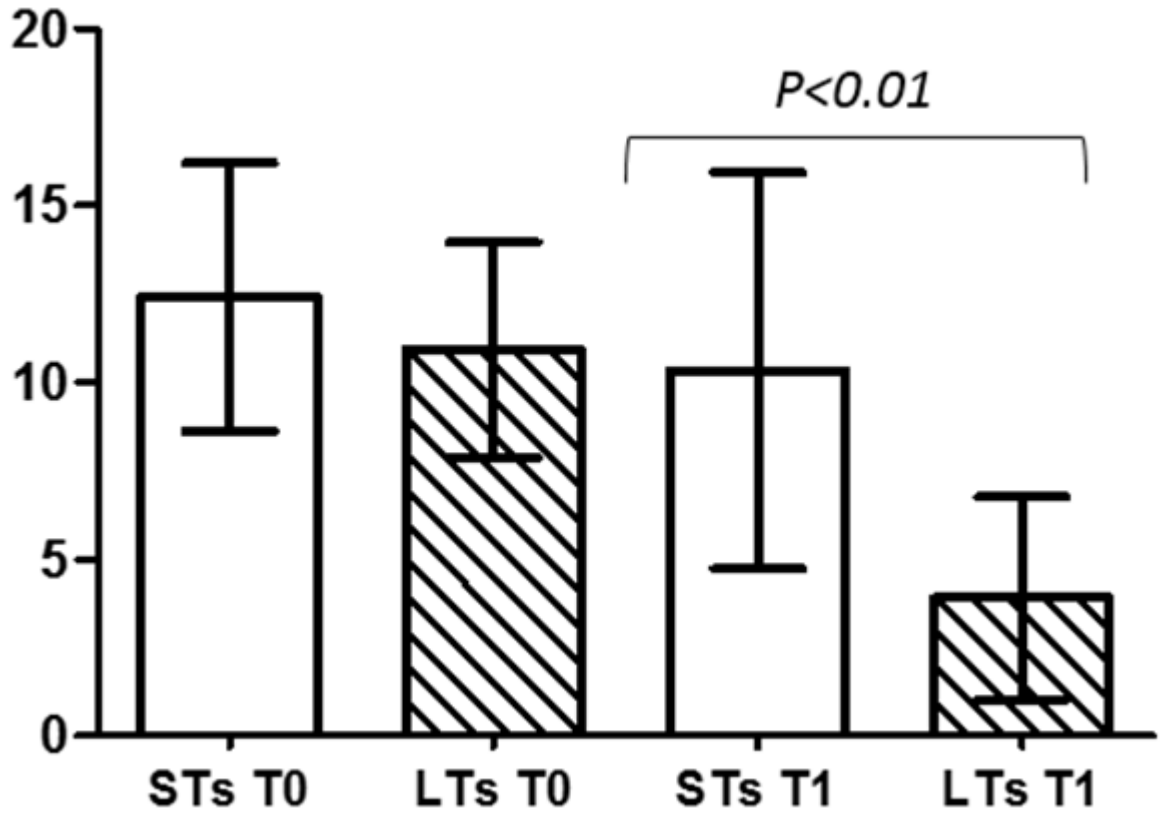
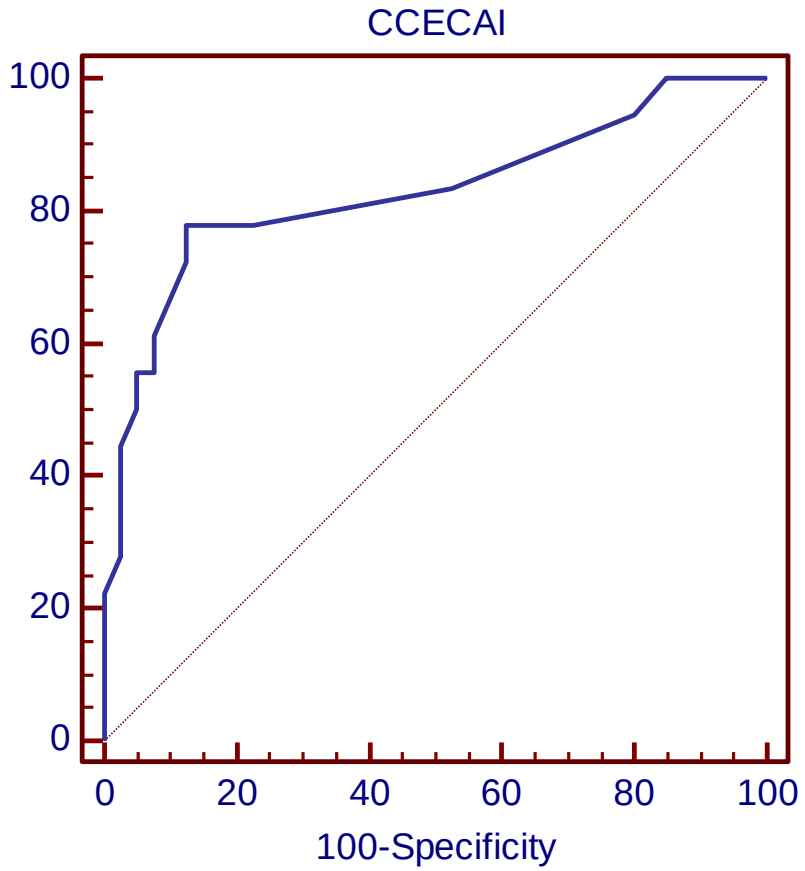


Figure 2. The receiver operating characteristic (ROC) curve used to select the optimum cut-off value of the variable CCECAI associated with survival to discriminate between short-term (ST) and long-term (LT) survivors.

Sensitivity: 77.8
Specificity: 87.5
Criterion: >5
Area under the ROC curve: 0.828
Standard error: 0.0672
95% Confidence interval: 0.706 to 0.914
Z statistic: 4.878
Significance level P <0.0001

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