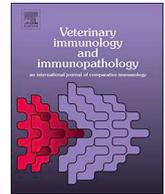




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Research paper

Mucosal expression of S100A12 (calgranulin C) and S100A8/A9 (calprotectin) and correlation with serum and fecal concentrations in dogs with chronic inflammatory enteropathy

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ABSTRACT

S100A12 and S100A8/A9 (calprotectin) are released from activated mononuclear cells and belong to the group of damage associated molecular patterns. Fecal S100A12 and S100A8/A9 concentrations have been suggested as biomarkers of intestinal inflammation in dogs with chronic inflammatory enteropathies (CIE). However, the mucosal cellular infiltrate in dogs with CIE is primarily lymphocytic-plasmacytic. Whether fecal S100A12 and S100A8/A9 levels reflect the number and/or activity of intestinal mucosal mononuclear cells, or whether these proteins are also produced by other cells has not been investigated. Thus, the aim of this study was to evaluate intestinal mucosal S100A12 and S100A8/A9 positivity and a potential relationship with the respective protein concentrations in serum and fecal samples in dogs with CIE.

Serum (single sample), fecal samples (from 3 consecutive days), and gastrointestinal tissue biopsies (i.e., stomach, duodenum, ileum, and colon) were evaluated from 21 dogs with CIE. Serum and fecal S100A12 and S100A8/A9 concentrations were measured by analytically validated in-house ELISAs. Tissue biopsies underwent routine histopathology and immunohistochemical evaluation for S100A12 and S100A8/A9 positivity (S100A12⁺ and S100A8/A9⁺, each recorded as positive cells/mm²).

S100A12⁺ and S100A8/A9⁺ cells were identified in all segments of the gastrointestinal tract, but were predominantly localized in the lamina propria (LP). Duodenal LP S100A12 positivity correlated statistically significantly with that in the stomach and ileum ($\rho = 0.66$ and 0.69 , both $p < 0.01$), but was inversely correlated with the severity of macrophage infiltration in the duodenum ($\rho = -0.47$, $p = 0.042$). Ileal LP S100A8/A9 positivity correlated positively with the extent of ileal neutrophil and macrophage infiltration ($\rho = 0.61$, $p = 0.047$). Fecal S100A12 concentrations strongly correlated with the number of S100A12⁺ cells along the entire gastrointestinal tract ($\rho = 0.76$, $p = 0.028$), whereas serum S100A12 concentrations were inversely correlated to colonic S100A12⁺ cell counts ($\rho = -0.50$, $p = 0.043$). Mucosal S100A8/A9⁺ cell counts were not associated with the corresponding fecal or serum S100A8/A9 concentrations.

These results suggest that the intestinal mucosa in dogs with CIE contains an increased number of activated (pro-inflammatory) phagocytes expressing and secreting the S100A12 protein, but the macrophage population

Abbreviations: CCECAI, canine chronic enteropathy clinical activity index; CIE, chronic inflammatory enteropathies; CRP, C-reactive protein; DAB, 3,3'-diaminobenzidine; DAMP, damage-associated molecular pattern; IBD, inflammatory bowel disease; IQR, interquartile ranges; LP, lamina propria; LPC, lymphoplasmacytic; MΦ, macrophages; RAGE, receptor for advanced glycation end products; SRE/IRE, steroid-/ immunosuppressant-responsive enteropathy; WSAVA, World Small Animal Veterinary Association

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seen on routine histopathology is predominantly mature (anti-inflammatory) with a reduced or absent expression of S100A12 and a normal or increased expression of S100A8/A9. However, the distribution of intestinal S100A8/A9 expression requires further study.

1. Introduction

Chronic inflammatory enteropathies (CIE) comprise an important group of diseases in dogs, the pathogenesis of which is complex and involves a dysregulated immune response (Dandrieux, 2016; Erdmann and Heilmann, 2017). The innate immunity appears to play a central role in the development of CIE in dogs (Jergens and Simpson, 2012; Heilmann and Allenspach, 2017).

The S100A12 protein (also referred to as calgranulin C) is a damage-associated molecular pattern (DAMP) molecule of the innate immune response (Foell et al., 2009; Goyette and Geczy, 2011) that is predominantly released from activated mononuclear cells (Goyette and Geczy, 2011; Heilmann et al., 2010; Heilmann et al., 2016a,b). S100A12 protein targets a number of different receptor proteins, including the receptor for advanced glycation end products (RAGE) (Hofmann et al., 1999), thus playing a central role in both innate and acquired immune responses (Gebhardt et al., 2008). In addition, S100A12 has several regulatory cellular functions (Hofmann et al., 1999; Pietzsch and Hoppmann, 2008). The concentration of S100A12 protein in fecal samples has been shown to be increased and to correlate with the severity of clinical signs, endoscopic lesions, and histologic inflammatory changes in dogs diagnosed with CIE (Heilmann et al., 2014a, 2016a; Heilmann and Steiner, 2018a). In addition, fecal S100A12 concentrations may have prognostic potential (Heilmann et al., 2016a; Heilmann and Steiner, 2018a).

Calprotectin, also referred to as the S100A8/A9 protein complex, belongs to the group of DAMP molecules as well and appears to be a useful fecal biomarker of intestinal inflammation in dogs (Heilmann et al., 2018b; Otoni et al., 2018). S100A8/A9 is expressed primarily by activated macrophages and neutrophils, but expression can also be induced in epithelial cells. S100A8/A9 is a ligand for Toll-like receptor-4, playing a role in acute and chronic inflammation (Vogl et al., 2007). Fecal S100A8/A9 concentrations were shown to be a good surrogate marker of disease severity and to predict the response to treatment in dogs with CIE (Grellet et al., 2013; Heilmann et al., 2018b; Otoni et al., 2018). These previous findings are interesting because the intestinal mucosal cellular infiltrate in dogs with CIE is predominated by lymphocytes, plasma cells, and eosinophils, whereas neutrophils represent only a small proportion of cells in most cases (Day et al., 2008; Washabau et al., 2010; Jergens and Simpson, 2012). The role of macrophages (MΦ) in CIE pathogenesis is not entirely clear (German et al., 2000; Wagner et al., 2018).

As no correlation between fecal S100A12 or S100A8/A9 concentrations and the site(s) of inflammatory lesions or the severity of MΦ/neutrophilic cellular infiltrates in the intestinal mucosa has been described, it remains unclear from which part of the intestine and from what cell population the S100A12 protein and the S100A8/A9 protein complex originate. Whether fecal S100A12 and/or S100A8/A9 concentrations reflect the number and/or activity of intestinal mucosal mononuclear cells, or whether these proteins are also produced by other cells has not been investigated. Thus, the aim of the current study was to test the possibility of an association between S100A12 and S100A8/A9 positivity of intestinal biopsy specimens and serum and fecal S100A12 and S100A8/A9 concentrations in dogs diagnosed with CIE.

2. Materials and methods

2.1. Sampling population

Materials from dogs (n = 21) diagnosed with chronic inflammatory

enteropathy according to the current World Small Animal Veterinary Association (WSAVA) guidelines (Washabau et al., 2010; Dandrieux, 2016; Erdmann and Heilmann, 2017) and included in a previous investigation (Heilmann et al., 2018b) were used for this study. Ethics approval was granted for the study (CRRC# 2009-06 and 2010-05, IACUC AUP# 2012-083), and written owner consent was obtained prior to patient enrollment. Dogs were only included if they had not undergone anti-inflammatory or immunosuppressive treatment.

A canine chronic enteropathy clinical activity index (CCECAI) score was calculated for all dogs included in the study to assess the severity of clinical signs at the time of diagnosis (Allenspach et al., 2007). This 9-parameter scoring system considers the following parameters: attitude and activity, appetite, frequency of vomiting, stool consistency and frequency of defecation, weight loss, serum albumin concentration, peripheral edema or ascites, and pruritus. The cumulative CCECAI score can range from 0 to 27 and is interpreted as clinically insignificant disease (cumulative score 0–3), mild disease (cumulative score of 4–5), moderate disease (cumulative score of 6–8), severe disease (cumulative score of 9–11), or very severe disease (score of ≥ 12) (Allenspach et al., 2007).

Follow-up information was available from 4 dogs, all of which required treatment with an anti-inflammatory and/or immunosuppressive drug and thus were determined to have steroid/immunosuppressant-responsive enteropathy (SRE/IRE) (Dandrieux, 2016). However, the possibility of an association between S100A12 and S100A8/A9 expression and the response to treatment could not be evaluated due to the small number of dogs from which follow-up data were available.

2.2. Sample collection and routine diagnostics

Whole blood and serum were collected from each dog as part of the routine diagnostic work-up, and was used for a routine hematology, serum biochemistry profile, and measurement of serum cobalamin, folate, and C-reactive protein (CRP) concentrations. Fecal samples (aliquots of approximately 1 g) were collected from 3 consecutive days prior to the collection of tissue biopsies, and were stored frozen until further analysis.

Sections of endoscopically-collected gastrointestinal tissue specimens (n = 19) or surgical biopsies (n = 2) were obtained from each dog and were submitted for routine histopathologic evaluation using the WSAVA Gastrointestinal Standardization grading system (Day et al., 2008). Morphologic lesions and inflammatory changes in the stomach, duodenum, ileum, and colon were graded on a 4-point scale (0 = normal, 1 = mild lesions, 2 = moderate lesions, and 3 = severe lesions) and cumulative lesion scores (i.e., the sum of individual lesion scores) were calculated.

2.3. Measurement of serum and fecal S100A12 and S100A8/A9 concentrations

Fecal specimens were extracted and analyzed for S100A12 and S100A8/A9 concentrations as previously described (Grützner et al., 2014; Heilmann et al., 2016b), and the same species-specific in-house sandwich ELISA tests were used for measurement of serum S100A12 and S100A8/A9. Briefly, fecal samples were diluted 1:5 in extraction buffer with proteinase inhibitors added, homogenized, and centrifuged, and supernatant fecal extracts were used for further analyses. Assay plates were incubated with rabbit polyclonal anti-canine S100A12 or anti-canine S100A8/A9 antibody, non-specific binding sites were

blocked, and then calibrator solutions, quality controls, assay blanks, and test samples were applied. After incubation with horseradish peroxidase-conjugated antibody (rabbit polyclonal anti-canine S100A12 or anti-canine S100A8/A9), the assay was developed, the absorbances

were measured in each well, and S100A12 or S100A8/A9 concentrations in test samples were determined by use of 5-parameter logistic curve fits. The S100A12 ELISA has a lower detection limit of 5 µg/L for serum and of 1 ng/g for fecal extracts (Heilmann et al., 2016a,b). The

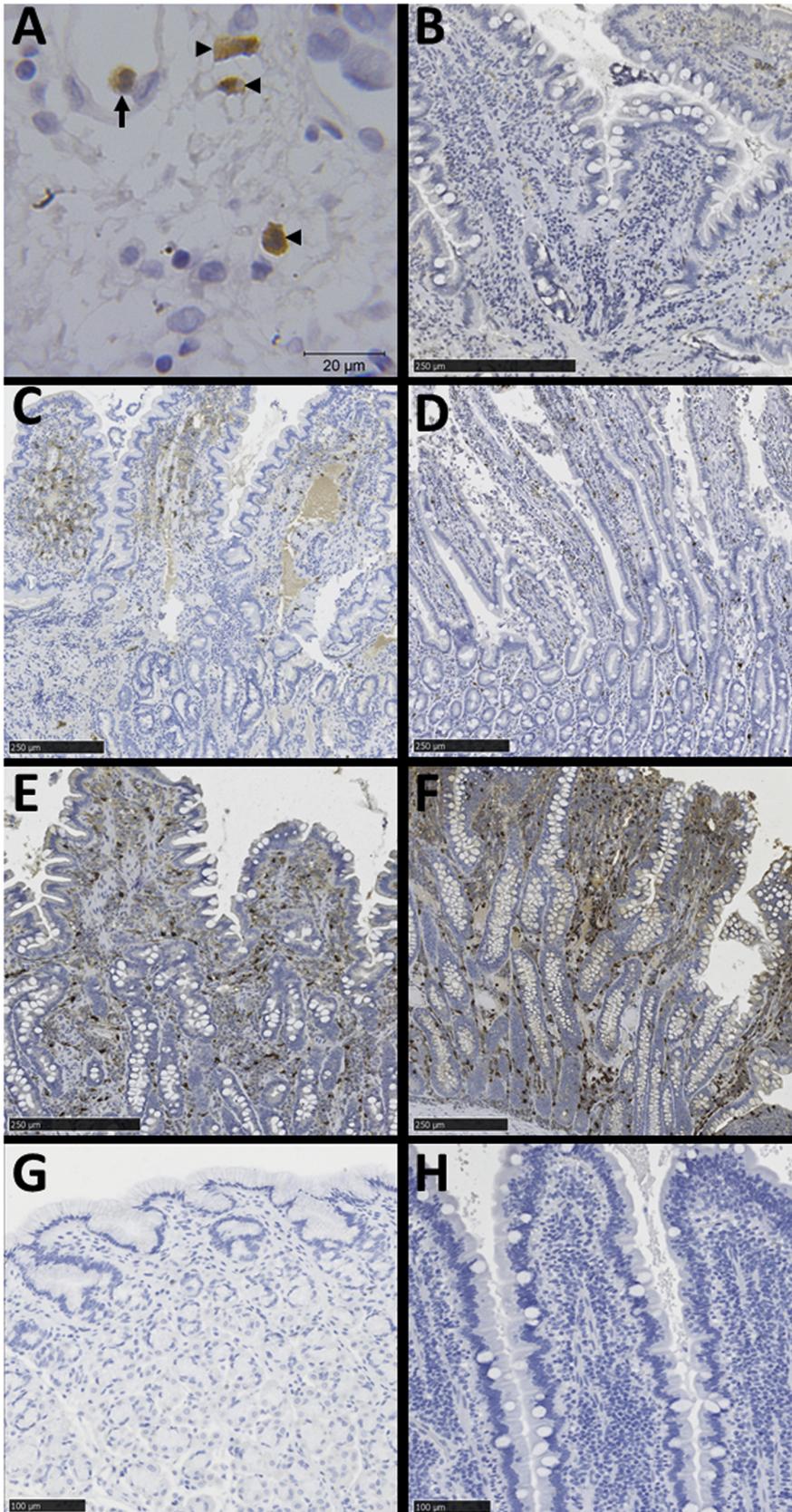


Fig. 1. S100A12 immunohistochemistry (IHC) of gastrointestinal tissue biopsies. (A) Only infiltrating cells staining positive for S100A12 (S100A12⁺ cells, arrow heads), but not intravascular S100A12⁺ cells (arrow), were counted and considered for analysis (protocol development). IHC evaluation of intestinal tissues from dogs with CIE (B–F) stained by use of the automated method shows varying numbers of intraepithelial (IE) and lamina propria (LP) S100A12⁺ cells: (B) duodenum with very few LP but no IE S100A12⁺ cells; (C) duodenum with a small number of LP but no IE S100A12⁺ cells; (D) ileum with moderate LP and small IE S100A12⁺ cell counts; (E) duodenum containing a moderate number of LP S100A12⁺ cells, but no IE S100A12⁺ cells; (F) ileum with a marked infiltrate of LP S100A12⁺ cells and also a moderate number of IE S100A12⁺ cells. (G) stomach, negative IHC control; (H) duodenum, negative IHC control.

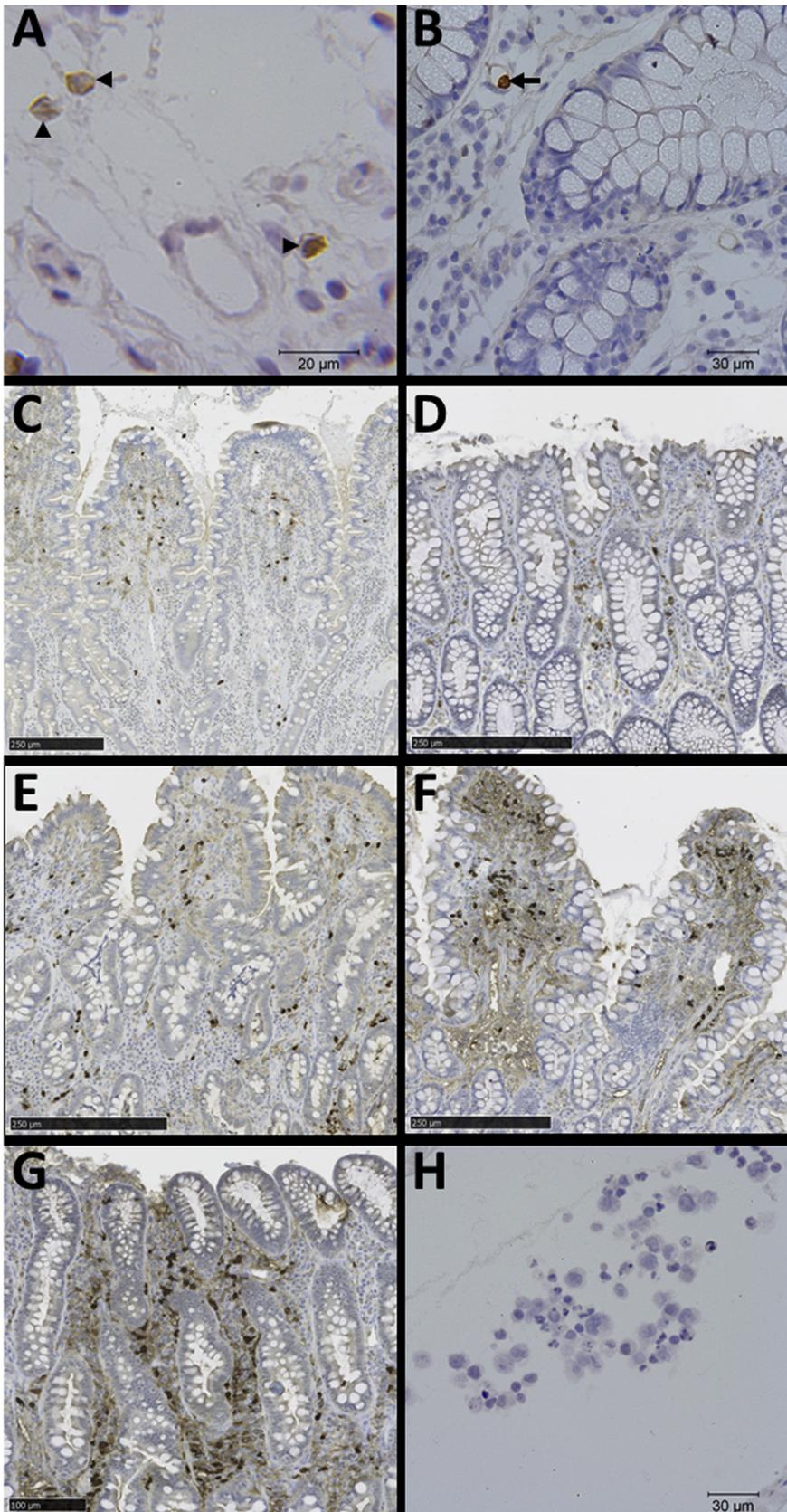


Fig. 2. S100A8/A9 immunohistochemistry (IHC) of gastrointestinal tissue biopsies. (A) Only infiltrating cells staining positive for S100A8/A9 (S100A8/A9⁺ cells, arrow heads), but not (B) intravascular S100A8/A9⁺ cells (arrow), were counted and considered for analysis (protocol development). IHC evaluation of intestinal tissues from dogs with CIE (C–G) stained by using the automated method shows varying numbers of intraepithelial (IE) and lamina propria (LP) S100A8/A9⁺ cells: (C) duodenum containing a small number of LP but no IE S100A8/A9⁺ cells; (D) colon with a small number of LP and occasional IE S100A8/A9⁺ cells; (E) duodenum containing a mild to moderate infiltrate of LP S100A8/A9⁺ cells and a small number of IE S100A8/A9⁺ cells; (F) ileum with moderate LP and small to moderate IE S100A8/A9⁺ cell counts; (G) colon with a moderate to marked infiltrate of LP S100A8/A9⁺ cells and a small number of IE S100A8/A9⁺ cells. (H) intestinal LP blood vessel, negative IHC control.

lower detection limit of the S100A8/A9 ELISA for serum is 0.3 mg/L and is 3.2 µg/g for fecal extracts (Heilmann et al., 2011b).

2.4. Immunohistochemistry of various tissue biopsy specimens (protocol development)

Specificity of the polyclonal antibodies generated against canine S100A12 (α -cA12) and canine S100A8/A9 (α -cA8/A9) that were used for immunohistochemistry analysis in this study were found to be specific in Western blot analysis (Heilmann et al., 2016b) as well as in immunoassays employing the same antibodies or antiserum (Heilmann et al., 2011a, 2014b). No immune cross-reactivity was observed between the two close homologues S100A8/A9 and S100A12 (Heilmann et al., 2011a, 2014b; Heilmann et al., 2016b).

Tissue biopsies were fixed in neutral buffered formalin (10%) for a minimum of 24 h at approximately 23 °C, embedded in paraffin wax, and the blocks were cut into 4 µm-sections. Following deparaffinization and rehydration, the biopsy sections were boiled in 0.01 M citrate buffer (pH 6.0) supplemented with 0.05% Tween for 45 min. Endogenous peroxidase activity was quenched by incubating the slides in 0.3% (v/v) hydrogen peroxide for 45 min at room temperature, and endogenous Fc-receptors were blocked by an incubation with 4% BSA in PBS (S100A12) or 2.5% normal horse serum in PBS (S100A8/A9) for 25 min at room temperature. After the tissue sections were incubated with the primary antibody (α -cA12: 250 µg/L; α -cA8/A9: 50 µg/L) for 1 h at room temperature, the secondary antibody (ImmPRESS™ Reagent Anti-Rabbit Ig; Vector Laboratories, Burlingame, CA, USA) was applied followed by an incubation for 30 min at room temperature. Sections where the primary antibody solution was replaced served as negative control tissue section. Slides were then thoroughly rinsed and positive staining indicated by 3,3'-diaminobenzidine (DAB), visualized as a brown product, and lightly counterstained with Mayer's hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) for visualization of the nuclei. Standard light microscopy (CX41 microscope; Olympus, Center Valley, PA, USA; and Insight FireWire 2 Megasample Color camera; Spot Imaging Solutions, Sterling Heights, MI, USA) at 40× magnification was used to evaluate positive staining cells in the tissue biopsies. A software package (Microscopy software v5.1, Spot Imaging Solutions) was used for image capture and processing.

2.5. Immunohistochemistry of gastrointestinal tissue biopsy samples (automated method)

Canine gastrointestinal biopsies fixed in 10% neutral-buffered formalin at room temperature for 24 h and embedded in paraffin, were then sectioned at 4 µm. After deparaffinization and rehydration, the slides were immersed in a citrate buffer. A pressure cooker (Decloaker; Biocare Medical, Concord, CA, USA) was used for antigen retrieval. The immunohistochemistry procedure was performed using a polymer detection kit on the IntelliPATH FLX® staining platform (Biocare Medical). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide, followed by incubation with IntelliPATH™ Background Punisher (Biocare Medical) to decrease non-specific protein background staining. The slides were incubated with the primary antibody (rabbit polyclonal α -cA12, 50 µg/L or rabbit polyclonal α -cA8/A9, 5 µg/L) for 1 h at room temperature. A negative serum replaced the primary antibody for the negative control slides. The polymer MACH 2™ anti-rabbit HRP (Biocare Medical) was then applied for 25 min. DAB was used to show sites of antigen-antibody interaction, and was indicated by a brown pigment. The sections were then counterstained with Mayer's hematoxylin. All slides were then converted to digital data using a whole slide scanner (NanoZoomer; Hamamatsu, Bridgewater, NJ, USA).

2.6. Gastrointestinal tissue immunohistochemistry analysis

Images were evaluated using the NDP.view2 software

(Hamamatsu). For S100A12 and S100A8/A9 quantification, the analysis of mucosal positivity was evaluated by separate assessment of two compartments: the epithelial layer and the lamina propria. Cell counts (positivity for S100A12 and for S100A8/A9) were performed by counting positive cells in 3 (S100A12) or 5 (S100A8/A9) high-power fields (1 mm² each) and calculating the mean number of positive cells in all fields (i.e., three or five fields, respectively) that were identified, whereby intravascular cells were not included into the cell counts (Figs. 1A and 2 A–B). All tissue sections were evaluated by a single board-certified veterinary pathologist (CG) together with two trainees (JN, JS), with all three investigators blinded to the identity, clinical and clinicopathological findings, and the histological diagnoses of the dogs.

2.7. Statistical analysis

Commercially available software packages were used for all statistical analyses (JMP® v13.0, SAS Institute, Cary, NC, USA; and GraphPad Prism® v7.0, GraphPad Software, San Diego, CA, USA). Normality of the data distribution was tested using a Shapiro-Wilk test. Summary statistics are reported as medians and ranges (or interquartile ranges, IQR). Mean fecal S100A12 (calgranulin C) and fecal S100A8/A9 (calprotectin) concentrations were calculated for the 3-day sample collection (Grützner et al., 2014; Heilmann et al., 2016b) and were considered for statistical analyses. Two-group comparisons were performed using a Wilcoxon rank sum test. Correlation analyses were performed by calculation of a Spearman ρ correlation coefficient. Statistical significance was set at $P < 0.05$, and trends were discussed using a $P < 0.10$.

3. Results

3.1. Sampling population

The clinical and clinicopathological findings of the dogs included in this study are summarized in Table 1. CCECAI scores ranged from 1 to 12 (median: 8). The most common clinical sign was diarrhea (85%), followed by hypo- or anorexia (65%), vomiting (55%), and weight loss (55%). Numbers of sections biopsied (endoscopically-collected specimens: 19, surgical specimens: 2) ranged from 2 to 4 (median: 4). Histological lesion scores ranged from 0 to 3 (median: 2). Numbers of biopsies evaluated for S100A12 positive (S100A12⁺) and S100A8/A9 positive (S100A8/A9⁺) cells are summarized in Tables 2 and 3, respectively. Hypoalbuminemia, shown to be a negative prognostic factor in canine CIE (Allenspach et al., 2007), was not associated with the severity of intestinal mucosal phagocyte infiltration (all $P > 0.05$).

3.2. S100A12 (calgranulin C) immunohistochemistry

S100A12⁺ cells were detected in all dogs evaluated in this study and in all segments of the gastrointestinal tract. S100A12 positivity was predominantly localized to the lamina propria, and individual dogs also showed S100A12⁺ staining within the epithelial layer (Table 2, Fig. 1). In biopsies of 8 of the 21 cases the submucosa could also be assessed and contained between 2–81 cells (duodenum: 5–32, median = 25, $n = 3$; ileum: 2–81, median = 32, $n = 8$; colon: 4–47, median = 36, $n = 4$) staining positive for S100A12. Positivity for S100A12 was associated with a cytoplasmic-membranous staining pattern.

There was no difference in lamina propria S100A12⁺ cell counts among the different segments (Table 2) of the gastrointestinal tract ($P = 0.833$). Numbers of lamina propria cells staining positive for S100A12 in the duodenum were correlated with the numbers of S100A12⁺ cells in the stomach ($\rho = 0.66$, $P = 0.003$) and the ileum ($\rho = 0.69$, $P = 0.002$); no correlations were seen for intraepithelial S100A12⁺ cells among the different segments of the gastrointestinal tract (all $P > 0.05$).

The severity of histologic lesions was not associated with the

Table 1

Patient characteristics, clinical findings, and clinicopathologic parameters in dogs with chronic inflammatory enteropathies (CIE) included in the study (n = 21).

Patient characteristic	Variable
Age in years, median (IQR)	5 (3–9)
Sex, male/female	12/9
Body weight in kg, median (IQR)	15.9 (7.2–26.4)
Breed, n (%)	
- pure-breed	18 (86%)
- mixed breed	3 (14%)
Number of sites biopsied, median (IQR)	4 (2–4)
Number of biopsies per site, median (IQR)	
- stomach ^a	10 (5–12)
- duodenum ^a	11 (7–19)
- ileum ^a	10 (4–14)
- colon ^b	12 (6–16)
Histologic lesion score, median (IQR)	2 (1–3)
Max. severity of histologic lesions, n (%)	
- none/minimal (histologic score = 0)	2 (9%)
- mild (histologic score = 1)	4 (19%)
- moderate (histologic score = 2)	10 (48%)
- severe (histologic score = 3)	5 (24%)
Clinical parameters	
CCECAI score ^c , median (IQR)	8 (4–9)
Clinical disease severity ^d , n (%)	
- mild (CCECAI score ≤ 5)	8 (40%)
- moderate (CCECAI score 6–8)	4 (20%)
- severe (CCECAI score 9–11)	7 (35%)
- very severe (CCECAI score ≥ 12)	1 (5%)
Clinicopathologic parameters	
Serum cobalamin in ng/L, median (IQR)	314 (266–680)
Serum folate in µg/L, median (IQR)	12.3 (9.1–16.2)
Serum albumin in g/dL, median (IQR)	2.7 (2.5–2.9)
Hypoalbuminemia ^e , n (%)	5 (24%)
Hypoalbuminemia severity ^e , n (%)	
- minimal (albumin 2.0–2.4 g/dL)	2 (9.5%)
- mild (albumin 1.5–1.99 g/dL)	2 (9.5%)
- moderate (albumin 1.2–1.49 g/dL)	1 (5%)
- severe (albumin < 1.2 g/dL)	0 (0%)
Serum and fecal biomarkers of inflammation	
Fecal calprotectin in µg/g ^f , median (IQR)	11.4 (0.5–60.6)
Fecal S100A12 in ng/g ^f , median (IQR)	381 (31–2448)
Serum CRP in mg/L, median (IQR)	9.1 (1.2–25.2)
Serum calprotectin in µg/L ^e , median (IQR)	5934 (5311–9887)
Serum S100A12 in µg/L ^e , median (IQR)	201 (148–248)

CCECAI: canine chronic enteropathy clinical activity index; CRP, C-reactive protein; IQR: interquartile range.

^a Available from n = 19 dogs.

^b Available from n = 18 dogs.

^c Documented in n = 20 dogs.

^d Documented in n = 7 dogs.

^e Documented in n = 8 dogs.

^f Measured in n = 13 dogs.

number of epithelial or lamina propria S100A12⁺ cells in any of the segments of the gastrointestinal tract (Table 2). There was an inverse correlation between the intensity of MΦ infiltration and numbers of lamina propria S100A12⁺ cells in the duodenum (Table 4), and numbers of MΦ also correlated between the duodenum and stomach ($\rho = 0.69$, $P = 0.001$). However, there was no correlation between the number of S100A12⁺ cells and infiltrating neutrophils in any of the segments. An inverse correlation was also detected between the intensity of lymphoplasmacytic (LPC) infiltration and numbers of epithelial S100A12⁺ cells in the duodenum, and the severity of lacteal dilation was inversely related to the numbers of lamina propria S100A12⁺ cells in the duodenum and ileum (Table 4).

Fecal S100A12 concentrations strongly correlated with the numbers of S100A12⁺ cells along the gastrointestinal tract, but there was a negative association between serum S100A12 concentrations and the numbers of S100A12⁺ cells in the colon (Table 5). No association of any parameter involving S100A12 with the CCECAI score was detected

(all $P > 0.05$). Significantly higher numbers of intraepithelial S100A12⁺ cells in the ileum were detected in dogs with hypoalbuminemia compared to normoalbuminemic dogs ($P = 0.019$); and a trend for lower lamina propria S100A12⁺ cell counts in all intestinal segments (sum) was observed in hypoalbuminemic dogs ($P = 0.078$).

3.3. S100A8/A9 (calprotectin) immunohistochemistry

S100A8/A9-positive (S100A8/A9⁺) cells were also identified in all segments of the gastrointestinal tract, with S100A8/A9 positivity being also predominantly localized to the lamina propria (Table 3, Fig. 2). In individual biopsies from 11 of the 13 dogs, S100A8/A9⁺ staining could also be assessed in the submucosa which contained between 0–28 S100A8/A9⁺ cells (duodenum: 0–21, median = 0, n = 5; ileum: 0–28, median = 3, n = 8; colon: 0–18, median = 2, n = 6). Staining for S100A8/A9 was also associated with a cytoplasmic-membranous staining pattern.

Numbers of lamina propria S100A8/A9⁺ cell counts were significantly lower in the stomach compared to the duodenum ($P = 0.057$), ileum ($P = 0.001$), and colon ($P = 0.003$); but no differences were detected among the intestinal segments (all $P > 0.05$) (Table 3). Numbers of lamina propria S100A8/A9⁺-positive cells were not correlated among any of the segments of the gastrointestinal tract evaluated (all $P > 0.05$), whereas intraepithelial S100A8/A9⁺ cells in the duodenum were correlated with the numbers of S100A8/A9⁺ cells in both the stomach ($\rho = 0.75$, $P = 0.020$) and ileum ($\rho = 0.64$, $P = 0.045$).

Epithelial or lamina propria S100A8/A9⁺ cells were not related to the severity of microscopic lesions in any segment of the gastrointestinal tract, but a trend for larger numbers of lamina propria S100A8/A9⁺ cells with more severe histologic lesions was seen in the ileum and colon (Table 3). The ileal lamina propria S100A8/A9⁺ cells count was correlated with the intensity of MΦ and also neutrophil infiltration (Table 4), with a trend for a correlation with the ileal cumulative inflammatory lesions score. Numbers of S100A8/A9⁺ cells did not correlate with that of infiltrating MΦ or neutrophils in any of the other segments or with any other structural or inflammatory criteria (all $P > 0.05$).

Serum and fecal S100A8/A9 (calprotectin) concentrations were not significantly correlated with the numbers of S100A8/A9⁺ cells along the gastrointestinal tract, but the number of dogs included in this part of the analysis were small (Table 5). Mucosal S100A8/A9 positivity was also not associated with the CCECAI score (Table 5). Significantly larger intraepithelial S100A8/A9⁺ cell counts in the duodenum were seen in hypoalbuminemic dogs than in normoalbuminemic patients ($P = 0.035$), with no differences observed in any other segments or layers (all $P > 0.05$). Numbers of S100A8/A9⁺ and S100A12⁺ cells were correlated only in the gastric lamina propria ($\rho = 0.81$, $P = 0.008$) but not in any other gastrointestinal segments or layers (all $P > 0.05$).

4. Discussion

In this study, mucosal expression of S100A12 and S100A8/A9 were evaluated and compared to the severity of clinical signs, histologic lesions, and also to the concentrations of S100A12 and S100A8/A9 in serum and fecal samples. To our knowledge, this is the first study investigating the spatial expression (i.e., the horizontal, vertical, cellular, and also intracellular) of S100A12 in the intestinal tract and also of intestinal S100A8/A9 expression by using a species-specific antibody in dogs with CIE. In addition, this study is the first to compare mucosal S100A12 and S100A8/A9 expression with the corresponding serum and fecal concentrations of these proteins.

An inverse relationship was found between duodenal lamina propria MΦ counts obtained during routine histopathological examination and S100A12⁺ cell counts in immunohistochemical analysis. This result was unexpected given the previous report of a positive correlation

Table 2
Gastrointestinal tissue S100A12 positivity in the dogs included in the study (n = 21).

Histologic lesion severity	N	Epithelial S100A12 positivity (cell counts)			Lamina propria S100A12 positivity (cell counts)		
		Range in all tissue biopsies	Mean, median (range)	<i>P</i> [#]	Range in all tissue biopsies	Mean, median (range)	<i>P</i> [#]
Stomach	19	0–2	0 (0–1)		1–223	61 (6–156)	
none or mild lesions	12	0–2	0 (0–1)	0.302	1–223	50 (6–156)	0.553
moderate or severe lesions	7	0–1	0 (0)		3–138	70 (12–113)	
Duodenum	19	0–3	0 (0–2)		8–368	44 (19–319)	
none or mild lesions	9	0–3	0 (0–2)	0.519	8–117	44 (19–97)	0.713
moderate or severe lesions	10	0–3	0 (0–2)		10–368	55 (21–319)	
Ileum	19	0–35	0 (0–24)		16–215	56 (22–200)	
none or mild lesions	4	0–3	0 (0–2)	0.399	17–100	74 (25–87)	0.881
moderate or severe lesions	15	0–35	0 (0–24)		16–215	44 (22–200)	
Colon	18	0–2	0 (0–1)		9–351	49 (15–297)	
none or mild lesions	10	0–2	0 (0–1)	0.935	9–351	48 (15–297)	0.689
moderate or severe lesions	8	0–2	0 (0–1)		12–260	50 (26–212)	

N: number of dogs.

[#] *P*-values for the comparison between dogs with none or mild histologic lesions and dogs with moderate or severe histologic lesions.

between S100A12 concentrations in small intestinal (duodenal) mucosal extracts and the infiltrating MΦ/neutrophil counts obtained during routine histopathological examination (Hanifeh et al., 2018). However, the results of the present study are consistent with our previous finding of a lack of correlation between fecal S100A12 concentration and the presence or severity of intestinal lamina propria phagocyte infiltration (Heilmann et al., 2014a, 2018b).

Together with the correlation between fecal S100A12 concentrations and the number of S100A12⁺ cells along the intestinal tract, this might suggest that the MΦ population seen on routine histopathology might be predominantly mature (tissue-resident anergic, anti-inflammatory) MΦ in which S100A12 expression is reduced or absent (Bujko et al., 2018), whereas the intestinal mucosa in dogs with CIE contains an increased amount of likely newly recruited (activated, pro-inflammatory) MΦ that cause chronic inflammation and express the S100A12 protein (Bain and Mowat, 2011; Nolte et al., 2017; Bujko et al., 2018). However, this hypothesis requires further investigation in dogs. This is also in line with a previous study showing decreased numbers of Iba-1⁺, MHC II⁺, CD163⁺, and CD204⁺ cells in the duodenum and a slight increase in the number of CD64⁺ cells (with an overall decrease in MΦ numbers) in all gastrointestinal segments of dogs with inflammatory bowel disease (IBD), supporting that pro-inflammatory MΦ play a role in the pathogenesis of canine IBD (Wagner et al., 2018). However, ileal biopsies were not evaluated in the study by Wagner et al. (2018). Another study in human patients showing 13% S100⁺ MΦ and 15% S100⁺ neutrophils in the inflamed mucosa of patients with IBD, but 51% S100⁺ MΦ and 33% S100⁺ neutrophils in

non-inflamed mucosa, with higher total numbers in IBD (Leach et al., 2007) is also consistent with the results of our study. Lack of a relationship of S100A12⁺ cell counts with histologic lesions in the colon contrasts the previous report of a correlation of canine S100A12 concentrations in colonic mucosal extracts with the severity of overall histologic lesions and epithelial injury in the colon (Hanifeh et al., 2018).

Strong S100A12⁺ staining of neutrophils and monocytes within the vasculature further supports that S100A12 expression is predominantly a feature of newly recruited cells. However, double-staining with additional macrophage-specific markers (e.g., Iba-1, CD64, CD163, CD204, or IL-10 production) would need to be performed to prove or disprove this hypothesis. Further investigation of the functional aspects of intestinal S100A12 expression in canine CIE (e.g., the possibility of an association with the RAGE/soluble RAGE axis or the expression of hypoxia-inducible factor-1α as a marker of tissue hypoxia) is also warranted. Lack of an association between the severity of clinical signs (i.e., CCECAI score) and lamina propria S100A12 positivity agrees with the lack of a relationship between the canine IBD activity index (CIBDAI) score and the presence of different MΦ populations (Wagner et al., 2018) or intestinal mucosal S100A12 concentrations (Hanifeh et al., 2018).

S100A8/A9 (calprotectin) is expressed in neutrophils and certain (but not all) MΦ infiltrating the intestinal mucosa of patients with IBD (Fukunaga et al., 2018). Macrophages have a central role in the intestinal protective immunity and maintenance of intestinal homeostasis. In healthy individuals, intestinal MΦ present an anti-

Table 3
Gastrointestinal tissue S100A8/A9 (calprotectin) positivity in the dogs included in the study (n = 13).

Histologic lesion severity	N	Epithelial S100A8/A9 positivity (cell counts)			Lamina propria S100A8/A9 positivity (cell counts)		
		Range in all tissue biopsies	Mean, median (range)	<i>P</i> [#]	Range in all tissue biopsies	Mean, median (range)	<i>P</i> [#]
Stomach	9	0–3	0 (0–2)		1–32	9 (7–14)	
none or mild lesions	5	0	0 (0)	0.131	1–19	9 (8–14)	0.900
moderate or severe lesions	4	0–3	1 (0–2)		2–32	11 (7–13)	
Duodenum	11	0–12	0 (0–3)		2–82	26 (9–61)	
none or mild lesions	4	0–1	0 (0)	0.327	2–42	21 (9–33)	0.185
moderate or severe lesions	7	0–12	0 (0–3)		3–82	33 (12–61)	
Ileum	11	0–14	0 (0–6)		3–193	37 (10–103)	
none or mild lesions	2	0	0 (0)	0.339	3–36	15 (10–20)	0.076
moderate or severe lesions	9	0–14	0 (0–6)		6–193	37 (13–103)	
Colon	10	0–40	2 (0–29)		6–129	36 (13–81)	
none or mild lesions	5	0–13	4 (0–8)	0.750	7–76	18 (13–43)	0.095
moderate or severe lesions	5	0–40	1 (0–29)		6–129	73 (16–81)	

N: number of dogs.

[#] *P*-values for the comparison between dogs with none or mild histologic lesions and dogs with moderate or severe histologic lesions.

Table 4

Correlation of S100A12 and S100A8/A9 positivity with histologic findings in canine CIE. Shown are the correlations among epithelial and the lamina propria S100A12⁺ [and S100A8/A9⁺] cell counts and the severity of morphologic and inflammatory histologic lesions in the stomach, duodenum, ileum, and colon in dogs with CIE.

Parameter	Spearman ρ correlation coefficient (<i>P</i> -value)			
	Epithelial	Lamina propria	Epithelial	Lamina propria
	S100A12 ⁺	S100A12 ⁺	S100A8/A9 ⁺	S100A8/A9 ⁺
correlated with	cell counts	cell counts	cell counts	cell counts
	Stomach (n=19)		Stomach (n=9)	
Stomach (composite score) [#]	-0.34 (0.153)	0.05 (0.852)	0.59 (0.092)	-0.09 (0.821)
Morphologic criteria (sum)	-0.05 (0.830)	-0.01 (0.984)	-0.12 (0.761)	-0.35 (0.350)
- Surface epithelial injury	-0.18 (0.471)	0.05 (0.841)	-0.37 (0.330)	0.06 (0.876)
- Gastric pit epithelial injury	0.44 (0.059)	-0.25 (0.300)	-0.19 (0.629)	-0.28 (0.466)
- Fibrosis/glandular nesting/MA	-0.18 (0.471)	-0.05 (0.841)	0.28 (0.460)	-0.42 (0.257)
Inflammatory criteria (sum)	-0.40 (0.102)	0.03 (0.898)	0.25 (0.526)	-0.29 (0.447)
- Intraepithelial lymphocytes	0.11 (0.642)	-0.03 (0.893)	0.01 (0.972)	-0.08 (0.835)
- Lamina propria LPC	-0.29 (0.229)	-0.06 (0.824)	-0.23 (0.560)	-0.49 (0.179)
- Lamina propria eosinophils	-0.25 (0.294)	0.11 (0.651)	0.77 (0.016)	-0.16 (0.688)
- Lamina propria neutrophils	N/A	N/A	N/A	N/A
- Lamina propria MΦ	-0.08 (0.742)	-0.26 (0.285)	N/A	N/A
- Lymphofollicular hyperplasia	-0.19 (0.457)	0.14 (0.597)	-0.28 (0.464)	0.11 (0.788)
	Duodenum (n=19)		Duodenum (n=11)	
Duodenum (composite score)	-0.35 (0.147)	-0.13 (0.598)	-0.23 (0.499)	0.21 (0.534)
Morphologic criteria (sum)	-0.33 (0.162)	-0.17 (0.482)	-0.01 (0.984)	0.27 (0.427)
- Villus stunting	-0.08 (0.754)	-0.02 (0.937)	0.43 (0.189)	0.46 (0.152)
- Epithelial injury	-0.15 (0.544)	0.08 (0.750)	-0.15 (0.663)	-0.40 (0.222)
- Crypt distension	-0.19 (0.441)	-0.06 (0.807)	-0.13 (0.703)	0.22 (0.526)
- Lacteal dilation	-0.35 (0.138)	-0.48 (0.036)	0.03 (0.927)	0.10 (0.781)
- Mucosal fibrosis	-0.10 (0.678)	-0.26 (0.286)	N/A	N/A
Inflammatory criteria (sum)	-0.35 (0.145)	0.08 (0.754)	-0.47 (0.143)	-0.09 (0.800)
- Intraepithelial lymphocytes	-0.14 (0.565)	-0.26 (0.283)	-0.29 (0.386)	-0.04 (0.911)
- Lamina propria LPC	-0.47 (0.045)	-0.20 (0.412)	-0.17 (0.629)	0.08 (0.823)
- Lamina propria eosinophils	-0.11 (0.645)	0.45 (0.051)	-0.03 (0.933)	-0.38 (0.246)
- Lamina propria neutrophils	-0.19 (0.442)	0.15 (0.554)	-0.22 (0.514)	0.08 (0.827)
- Lamina propria MΦ	-0.15 (0.545)	-0.47 (0.042)	0.38 (0.249)	0.39 (0.233)
	Ileum (n=19)		Ileum (n=11)	
Ileum (composite score)	-0.52 (0.024)	-0.20 (0.423)	0.15 (0.661)	0.40 (0.218)
Morphologic criteria (sum)	-0.48 (0.037)	-0.29 (0.232)	0.04 (0.912)	0.21 (0.545)
- Villus stunting	-0.26 (0.290)	0.01 (0.972)	0.29 (0.385)	0.11 (0.746)
- Epithelial injury	-0.15 (0.546)	0.02 (0.933)	0.30 (0.377)	0.16 (0.632)
- Crypt distension	-0.37 (0.121)	-0.09 (0.702)	-0.08 (0.819)	0.24 (0.469)
- Lacteal dilation	-0.23 (0.348)	-0.49 (0.033)	-0.23 (0.493)	-0.10 (0.769)
- Mucosal fibrosis	N/A	N/A	N/A	N/A
Inflammatory criteria (sum)	-0.45 (0.051)	-0.09 (0.730)	0.42 (0.208)	0.54 (0.088)
- Intraepithelial lymphocytes	-0.33 (0.164)	-0.27 (0.262)	-0.06 (0.856)	0.09 (0.796)
- Lamina propria LPC	-0.25 (0.300)	-0.22 (0.369)	-0.23 (0.505)	0.18 (0.595)
- Lamina propria eosinophils	-0.34 (0.158)	0.30 (0.218)	0.32 (0.341)	0.21 (0.541)
- Lamina propria neutrophils	-0.12 (0.633)	-0.15 (0.545)	0.61 (0.047)	0.61 (0.047)
- Lamina propria MΦ	-0.15 (0.546)	-0.19 (0.436)	0.61 (0.047)	0.61 (0.047)
	Colon (n=18)		Colon (n=10)	
Colon (composite score)	-0.10 (0.685)	-0.07 (0.778)	-0.12 (0.744)	0.35 (0.320)
Morphologic criteria (sum)	-0.16 (0.537)	-0.23 (0.362)	-0.16 (0.660)	0.08 (0.836)
- Epithelial injury	-0.22 (0.388)	-0.13 (0.613)	-0.27 (0.459)	0.26 (0.466)
- Goblet cell loss or hyperplasia	-0.07 (0.779)	0.01 (0.983)	0.16 (0.666)	0.38 (0.276)
- Crypt dilation and distortion	0.04 (0.887)	-0.29 (0.244)	-0.24 (0.511)	-0.17 (0.639)
- Mucosal fibrosis and atrophy	-0.19 (0.457)	-0.14 (0.582)	-0.18 (0.625)	-0.06 (0.874)
Inflammatory criteria (sum)	0.34 (0.892)	0.04 (0.876)	-0.10 (0.783)	0.27 (0.460)
- Intraepithelial lymphocytes	0.22 (0.389)	0.15 (0.565)	-0.18 (0.629)	-0.03 (0.935)
- Lamina propria LPC	0.00 (1.000)	0.12 (0.631)	-0.22 (0.544)	0.18 (0.619)
- Lamina propria eosinophils	0.14 (0.589)	0.26 (0.306)	0.41 (0.243)	-0.03 (0.929)
- Lamina propria neutrophils	-0.09 (0.735)	-0.30 (0.220)	-0.18 (0.625)	0.06 (0.874)
- Lamina propria MΦ	-0.13 (0.622)	-0.36 (0.140)	-0.18 (0.625)	0.06 (0.874)

LPC: lymphocytes/plasma cells; MA: mucosal atrophy; MΦ: macrophages; N/A: not applicable; [#]lesions evaluated for fundus and antrum (combined). Cell counts highlighted in grey indicate a statistically significant correlation (*P* < 0.05), those cells highlighted in light grey indicate a trend for a significant correlation (*P* < 0.1).

Table 5

Correlation of mucosal S100/calgranulin positivity with serum and fecal protein concentrations and with the severity of clinical signs. Shown are the relationships between the numbers of gastrointestinal lamina propria (LP) S100A12⁺ [and S100A8/A9⁺] cells and the corresponding S100A12 [and S100A8/A9] concentrations in serum and feces as well as the clinical disease activity (CCECAI) score in dogs with CIE in this study.

Parameter	correlated with	Spearman ρ correlation coefficient (<i>P</i> -value)		
		serum S100A12 concentration	fecal S100A12 concentration [#]	CCECAI score
LP S100A12 ⁺ cells, all GI segments (sum) [§]		n=14 -0.02 (0.935)	n=8 0.76 (0.028)	n=14 -0.39 (0.163)
LP S100A12 ⁺ cells, intestinal segments (sum) [†]		n=15 -0.11 (0.685)	n=9 0.65 (0.058)	n=15 -0.39 (0.149)
LP S100A12 ⁺ cells, small intestine (sum) [‡]		n=17 0.03 (0.907)	n=11 0.15 (0.670)	n=16 -0.18 (0.511)
LP S100A12 ⁺ cells, large intestine (sum)		n=17 -0.50 (0.043)	n=12 0.24 (0.457)	n=18 -0.46 (0.053)
Parameter	correlated with	serum S100A8/A9 concentration	fecal S100A8/A9 concentration [#]	CCECAI score
LP S100A8/A9 ⁺ cells, all GI segments (sum) [§]		n=5 -0.50 (0.391)	n=6 0.49 (0.329)	n=6 -0.26 (0.618)
LP S100A8/A9 ⁺ cells, intestinal segments (sum) [†]		n=6 -0.54 (0.266)	n=7 0.36 (0.432)	n=7 -0.29 (0.531)
LP S100A8/A9 ⁺ cells, small intestine (sum) [‡]		n=6 -0.20 (0.704)	n=8 0.60 (0.117)	n=10 -0.15 (0.673)
LP S100A8/A9 ⁺ cells, large intestine (sum)		n=7 -0.54 (0.215)	n=9 -0.22 (0.576)	n=9 0.07 (0.862)

CCECAI: canine chronic enteropathy clinical activity index; GI: gastrointestinal, LP: lamina propria; [§]calculated only when stomach, duodenum, ileum, and colon were evaluated; [#]3-day mean concentration; [†]duodenum, ileum, and colon combined; [‡]duodenum and ileum combined. Cell counts highlighted in grey indicate a statistically significant correlation ($P < 0.05$), those cells highlighted in light grey indicate a trend for a significant correlation ($P < 0.1$).

inflammatory phenotype, whereas intestinal inflammation is commonly associated with an increased recruitment of blood monocytes or activation of intestinal resident cells differentiating into M Φ displaying a pro-inflammatory phenotype (Tamoutounour et al., 2012; Nolte et al., 2017; Wagner et al., 2018). In people, expression of the S100A8/A9 protein complex (L1 antigen) was shown to be restricted to newly recruited (immature) pro-inflammatory M Φ , whereas expression of L1 is down-regulated and eventually lost during M Φ maturation (Zwadlo et al., 1988). Thus, an increase of L1⁺ M Φ in the intestinal lamina propria suggests an influx of monocytes to the inflamed mucosa. In contrast to S100A12, however, S100A8/A9⁺ cell counts were positively correlated with the severity of M Φ and neutrophil infiltration in the ileum (but not the duodenum) in this study. While this is in contrast with the depletion of intestinal lamina propria anti-inflammatory M Φ seen in dogs with IBD (Wagner et al., 2018), it agrees with the correlation between the numbers of L1⁺, CD204⁺, and CD163⁺ cells and the WSAVA histopathology score (Wagner et al., 2018) and also with the results of another study that found higher numbers of MHC II⁺ and L1⁺ cells in the duodenum of dogs with IBD by using a computer-assisted morphometry for cell counting (German et al., 2000). However, both studies did not include evaluation of ileal biopsies and the findings in duodenal versus ileal biopsies were shown to rarely reflect each other (Procoli et al., 2013). Also, the interpretation of M Φ phenotypes as well as their differentiation from dendritic cells is difficult and the recruitment of proinflammatory M Φ in canine IBD is still a matter of debate (German et al., 2000; Wagner et al., 2018). Furthermore, specificity and/or sensitivity of the monoclonal L1-antibody MAC387 used in previous immunohistochemistry studies (German et al., 2000; Wagner et al., 2018) for the canine calprotectin protein complex might be lower than for the human counterpart as shown in a previous Western blot analysis (Heilmann et al., 2008). An alternative explanation might be that the S100A8/A9 protein or the protein complex detected by the L1-Ag-antibody has species-specific functions. Also, the functional implications of increased intestinal S100A8/A9 expression in canine CIE (e.g., a possible link to Toll-like receptor 4 expression and/or responsiveness) remain unknown and require further research.

For the purpose of this study, only cells staining clearly positive for S100A12 or S100A8/A9 were counted, but there was also some staining of both in the surrounding tissue without any clear cellular origin (Figs. 1 and 2). It is presumed that this reflects S100A12 and S100A8/A9 protein that has been secreted into the extracellular space by activated phagocytes, which is consistent with the secretion and localization of S100/calgranulins to the extracellular matrix detected by Leach et al. (2007) and also with both S100A12 and S100A8/A9 being detected and measured in fecal samples. This is further supported by the finding that the concentration of S100A12 protein in fecal samples correlated with the number of S100A12⁺ cells along the gastrointestinal tract, and it may also explain the differences between the findings in our current study and the results for mucosal S100A12 evaluated using tissue extracts (Hanifeh et al., 2018). A similar correlation was not detected for S100A8/A9, but this might also be attributed to the small number of dogs in which fecal S100A8/A9 could be determined.

A slightly unequal vertical distribution of both S100A12⁺ and S100A8/A9⁺ cells was noted in this study (Figs. 1 and 2), with higher cell counts in the villus area than the epithelium, crypt area, or sub-mucosal layer; but the different areas were not separately evaluated and statistically compared. This agrees with the vertical distribution of M Φ (except for CD64⁺ cells) seen in the study by Wagner et al. (2018) where a larger cell count was seen in the area of the villi than in the area of the crypts. No specific horizontal distribution pattern of S100A12⁺ cells was seen in this study, which also agrees with previous findings for intestinal M Φ (Wagner et al., 2018). However, there were significantly less lamina propria S100A8/A9⁺ cells in the stomach compared to all other gastrointestinal segments evaluated in this study. This could be explained by the inconsistent involvement of the stomach in canine CIE (Allenspach et al., 2018).

The lack of an association of the negative prognostic factor hypoalbuminemia (Allenspach et al., 2007) with the number of intestinal lamina propria neutrophils and intraepithelial lymphocytes found in this study agrees with a previous study in dogs showing absence of such an association in a multivariate model (Wennogle et al., 2017).

We acknowledge that this study had some limitations. First, a control group of healthy dogs was not included in this study. Hence, the possibility of a difference in S100A12⁺ and S100A8/A9⁺ cell counts could not be compared between dogs with CIE and healthy control dogs. This warrants further investigation in light of the finding that S100A12 concentrations in duodenal and colonic mucosal extracts were higher in dogs with CIE compared to healthy controls in a previous study (Hanifeh et al., 2018). Specific staining to further characterize the cells expressing S100A12 and/or S100A8/A9 were not performed. Thus, no clear statement can be made as to the expression of these proteins in neutrophils, dendritic cells, or different types of MΦ in canine CIE. Third, due to this study presenting a first evaluation of the S100A12 and S100A8/A9 expression at the mucosal level in dogs with CIE, a more conservative method to statistically correct a possible alpha-inflation (e.g., Bonferroni correction) was not applied. Further, the sample size for evaluation of the correlation between mucosal S100A8/A9 positivity and fecal S100A8/A9 concentrations was small. Thus, a type II error for finding no significant correlations cannot be excluded. And lastly, outcome was not evaluated in this study. Thus, no conclusion can be drawn as for the prognostic values of increased gastrointestinal mucosal S100A12⁺ and S100A8/A9⁺ cell counts.

5. Conclusion

In summary, the findings of this study support that S100A12 and S100A8/A9 as well as phagocyte populations (especially MΦ) play a role in the pathogenesis of canine IBD. The intestinal mucosal expression patterns of S100A12 and S100A8/A9 and their correlation with inflammatory (especially phagocytic) infiltrates appear to differ. Additional studies are warranted to further characterize the population of cells expressing S100A12 and/or S100A8/A9, to determine the functional implications of the differential expression of these proteins, and to evaluate whether intestinal lamina propria MΦ play a role in the breakdown of intestinal homeostasis and chronic mucosal inflammation.

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