

Fecal calprotectin concentrations in adult dogs with chronic diarrhea

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Objective—To evaluate fecal calprotectin concentrations in healthy dogs and dogs with chronic diarrhea, to identify cutoff values for fecal calprotectin concentrations for use in differentiating dogs with chronic diarrhea and a canine chronic enteropathy clinical activity index (CCECAI) < 12 from dogs with chronic diarrhea and a CCECAI ≥ 12, and to evaluate the association between histologic evidence of intestinal mucosal changes and fecal calprotectin concentrations in dogs with chronic diarrhea.

Sample—Fecal samples from 96 adult dogs (27 dogs with chronic diarrhea and 69 healthy control dogs).

Procedures—Severity of clinical signs was evaluated on the basis of the CCECAI scoring system. Endoscopy was performed in all dogs with chronic diarrhea, and mucosal biopsy specimens were evaluated histologically. Fecal calprotectin concentration was quantified via radioimmunoassay.

Results—Fecal calprotectin concentrations were significantly higher in dogs with chronic diarrhea than in healthy control dogs. Fecal calprotectin concentrations were also significantly higher in dogs with a CCECAI ≥ 12, compared with concentrations for dogs with a CCECAI between 4 and 11. Fecal calprotectin concentrations were significantly higher in dogs with chronic diarrhea associated with histologic lesions, compared with concentrations in control dogs, and were significantly correlated with the severity of histologic intestinal lesions. Among dogs with chronic diarrhea, the best cutoff fecal calprotectin concentration for predicting a CCECAI ≥ 12 was 48.9 µg/g (sensitivity, 53.3%; specificity, 91.7%).

Conclusions and Clinical Relevance—Fecal calprotectin may be a useful biomarker in dogs with chronic diarrhea, especially dogs with histologic lesions. (*Am J Vet Res* 2013;74:706–711)

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ABBREVIATIONS

CCECAI	Canine chronic enteropathy clinical activity index
CI	Confidence interval
IBD	Inflammatory bowel disease
ROC	Receiver operating characteristic
WSAVA	World Small Animal Veterinary Association

IBD are considered to be the most common chronic enteropathies in dogs.^{1–3} A clinical diagnosis of IBD requires chronic (ie, > 3 weeks' duration) signs of gastrointestinal tract disease, histologic evidence of mucosal inflammation, exclusion of other causes of gastroenterocolitis, and an inadequate response to therapeutic trials (ie, dietary, antimicrobial, or anthelmintic treatment).⁴ Histologic changes in the absence of these criteria do not allow clinicians to establish a diagnosis of idiopathic IBD. Also, histologic changes do not reflect the clinical severity of chronic enteropathies and do not aid in suggesting the optimal thera-

peutic strategy for a specific patient.^{5,6} The clinical severity of intestinal disease and response to treatment currently are assessed with clinical scoring systems, such as the canine IBD activity index and the CCECAI.^{5,7} Unfortunately, these scoring systems are based partially on a subjective assessment of clinical signs.⁷ More objective methods, such as biomarkers for inflammation, may be useful to assess disease severity in dogs with chronic enteropathies.

In human medicine, several biomarkers are used to limit the need for invasive diagnostic testing and to aid in evaluation of the progression of intestinal inflammation and therapeutic response. Calprotectin, a heterodimeric protein complex, which is present in neutrophils, monocytes, and reactive macrophages, is one of these markers.⁸ Fecal calprotectin concentrations are higher in human patients with Crohn's disease or ulcerative colitis, compared with concentrations in healthy control humans.^{9–15} Moreover, there is a good correlation between fecal calprotectin concentrations and disease activity, as determined by endoscopic and histologic scoring of biopsy specimens.^{16–18}

Significantly higher serum calprotectin concentrations have been reported in dogs with idiopathic IBD.¹⁹ However, studies to evaluate fecal calprotectin concentrations in dogs with chronic diarrhea have not been conducted. Therefore, the purpose of the study reported here was to measure calprotectin concentrations in feces of healthy dogs and dogs with chronic diarrhea, to identify cutoff values for the differentiation of dogs with chronic diarrhea associated with a CCECAI < 12 from dogs with chronic diarrhea associated with a CCECAI ≥ 12, and to evaluate a possible association between histologic intestinal mucosal lesions and fecal calprotectin concentrations in dogs with chronic diarrhea.

Materials and Methods

Dogs—Ninety-six adult dogs (69 healthy control dogs that had no signs of gastrointestinal disease and 27 dogs with chronic diarrhea) were included in the study. The study protocol was reviewed and approved by the Royal Canin Internal Ethics Committee. The authors adhered to Standards for Reporting of Diagnostic Accuracy recommendations.^{20,21}

Each of the 69 control dogs was assessed as healthy on the basis of history, physical examination findings, and an evaluation of fecal quality (feces was formed but not hard). Control dogs comprised 15 mixed-breed dogs, 11 German Shepherd Dogs, 5 Rottweilers, 3 Golden Retrievers, 3 Blue Heelers, 2 Border Collies, 2 Boston Terriers, 2 Dachshunds, 2 Great Pyrenees, 2 Weimaraners, 2 West Highland White Terriers, and 1 each of Bloodhound, Chihuahua, French Bulldog, Jack Russell Terrier, Miniature Schnauzer, Redbone Coonhound, and Yorkshire Terrier; breed was not defined for 13 dogs.

Twenty-seven dogs evaluated at the Cerisioz Veterinary Clinic, St Priest, France, between July and December 2010 because of a history of chronic (at least 3 weeks' duration) diarrhea were prospectively enrolled in the study. The initial diagnostic evaluation for all these

dogs included a serum biochemical analysis, urinalysis, fecal parasitological evaluation, abdominal ultrasonographic examination, and gastrointestinal endoscopy with endoscopic collection of tissue biopsy specimens. Dogs with chronic diarrhea comprised 3 Rottweilers, 3 Yorkshire Terriers, 3 mixed-breed dogs, 2 Akitas, 2 Bernese Mountain Dogs, 2 English Cocker Spaniels, 2 German Shepherd Dogs, and 1 each of Belgian Malinois, Boxer, Cane Corso, Cavalier King Charles Spaniel, English Bulldog, French Bulldog, Golden Retriever, Great Dane, Labrador Retriever, and Shih Tzu.

Clinical activity index—Physical examination was performed on all dogs with chronic diarrhea, and the severity of clinical signs was evaluated on the basis of the CCECAI scoring index.⁵ The CCECAI scoring system is based on the following 9 variables that are commonly altered in dogs with chronic enteropathies: attitude and activity, appetite, vomiting, fecal consistency, defecation frequency, weight loss, serum albumin concentration, presence of ascites and peripheral edema, and pruritus. For the CCECAI, a cumulative score ≥ 12 has been found to be the best predictor for a negative outcome (death because of refractoriness to treatment).⁵ This cutoff value was used to evaluate the relationship between the CCECAI and fecal calprotectin concentrations.

Endoscopy and histologic evaluation—Gastro-duodenoscopy was performed in the 27 dogs with chronic diarrhea. When clinical signs were suggestive of disease of the colonic portion of the gastrointestinal tract, gastroduodenoscopy followed by colonoscopy (n = 6 dogs) was performed. All endoscopic procedures were performed by a veterinarian board-certified in veterinary internal medicine (PL). Mucosal biopsy specimens were collected during endoscopy and were evaluated by a board-certified veterinary pathologist (MJD) who was unaware of the clinical data, endoscopy results, and fecal calprotectin concentrations. A scoring system developed by the WSAVA Gastrointestinal Standardization Group²² was used to score histologic findings. As recommended by the WSAVA,⁴ the veterinary pathologist reported the total number of tissue samples submitted and the quality of those samples (ie, inadequate, marginal, or adequate). In accordance with a recommendation from another study,²³ histologic results were only considered for dogs from which at least 6 adequate duodenal or colonic samples were obtained. Histologic changes were classified as mild, moderate, or severe in accordance with the WSAVA scoring system²²; scores ranged from 0 (no histologic change) to 9 (severe histologic changes). Associations between the severity of the histologic changes and fecal calprotectin concentrations were evaluated on the basis of the WSAVA classification system.

Fecal calprotectin assay—Fecal samples (2 to 10 g of feces) used for the evaluation of canine calprotectin concentrations were collected immediately before the endoscopic procedure from dogs with chronic diarrhea and after spontaneous defecation from healthy dogs. All fecal samples were stored frozen at –20°C until analysis. Fecal samples were extracted, and cal-

protectin concentrations were quantified as described elsewhere.²⁴

Statistical analysis—Statistical analyses were performed with statistical software.^a All data were tested for normality by use of the Shapiro-Wilk test. Fecal calprotectin concentrations were not normally distributed, so these data were reported as median and range values. A Mann-Whitney *U* test or Kruskal-Wallis test was used, depending on the number of groups considered. Correlations between the severity of histologic lesions and fecal calprotectin concentrations and between the severity of histologic lesions and the CCECAI were evaluated with the Spearman rank correlation test. Significance was set at $P < 0.05$ for all analyses. Analysis of an ROC curve was used to select the cutoff value that allowed the best differentiation between dogs with a CCECAI < 12 and dogs with a CCECAI ≥ 12 . Sensitivity and specificity were estimated.

Results

Fecal calprotectin concentrations and clinical signs—The median fecal calprotectin concentration was significantly ($P = 0.002$) higher (3.2 times as high) for the 27 dogs with chronic diarrhea (median, 35.1 $\mu\text{g/g}$; range, 2.9 to 2,093.9 $\mu\text{g/g}$) than for the 69 healthy control dogs (median, 11 $\mu\text{g/g}$; range, 2.9 to 109.8 $\mu\text{g/g}$; Figure 1). Dogs with chronic diarrhea had a median CCECAI of 12 (range, 4 to 18); 12 dogs had a CCECAI < 12 , and 15 dogs had a CCECAI ≥ 12 . In the 15 dogs with chronic diarrhea and a CCECAI ≥ 12 , the fecal calprotectin concentration (median, 60.3 $\mu\text{g/g}$; range, 7.8 to 2,093.9 $\mu\text{g/g}$) was significantly ($P = 0.025$) higher (4.3 times as high) than the fecal calprotectin concentration (median, 14 $\mu\text{g/g}$; range, 2.9 to 111 $\mu\text{g/g}$) for the 12 dogs with chronic diarrhea and a CCECAI < 12 and was also significantly ($P < 0.001$) higher (5.5 times as high) than the fecal calprotectin concentration (median, 11 $\mu\text{g/g}$; range, 2.9 to 109.8 $\mu\text{g/g}$) for the 69 healthy control dogs. Fecal calprotectin concentrations of dogs with chronic diarrhea and a CCECAI < 12 did not differ significantly ($P = 0.25$) from concentrations in healthy control dogs.

Cutoff value for fecal calprotectin concentration—The optimal cutoff value of the fecal calprotectin concentration for use in differentiating dogs with severe clinical disease (CCECAI ≥ 12) from dogs with mild to

moderate clinical disease (CCECAI < 12) on the basis of an ROC curve was 48.9 $\mu\text{g/g}$. This cutoff value had a sensitivity of 53.3% (95% CI, 30.2% to 75.1%), specific-

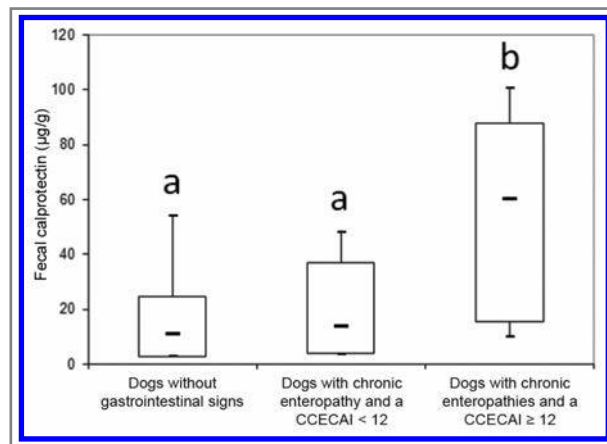


Figure 1—Box-and-whisker plot of fecal calprotectin concentrations in 69 healthy control dogs and 27 dogs with chronic diarrhea (12 with a CCECAI < 12 and 15 with a CCECAI ≥ 12). Each box represents the first to third quartiles (25th to 75th percentiles), the bar in each box represents the median, and the whiskers represent the first to ninth decile (10th to 90th percentiles). ^{a,b}Values with different letters differ significantly ($P < 0.05$).

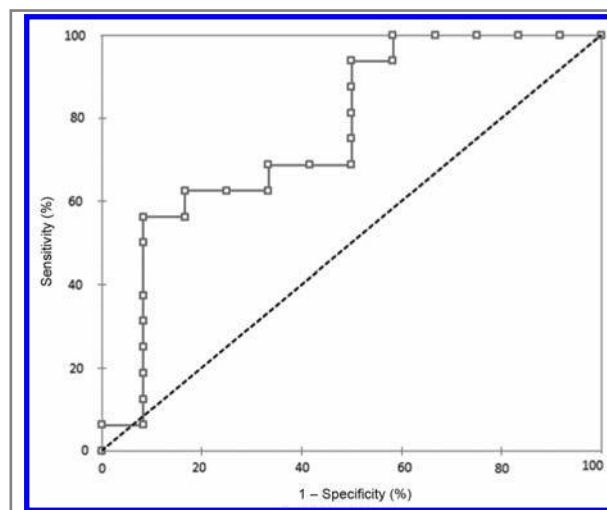


Figure 2—The ROC curve for fecal calprotectin concentrations used to differentiate between 12 dogs with chronic diarrhea and mild to moderate clinical disease (CCECAI < 12) and 15 dogs with chronic diarrhea and severe clinical disease (CCECAI ≥ 12). The area under the curve was 0.756 (95% CI, 0.579 to 0.9331). Notice the line of equality (dashed line).

Table 1—Histologic lesions observed in intestinal tissue biopsy specimens collected from 14 dogs with chronic diarrhea.

Localization of lesion	Diagnosis	No. of dogs	Intestinal lesion score*
Duodenum and jejunum	No inflammatory infiltration	5	0
	Mild lymphocytic-plasmacytic enteritis	5	2–4
	Moderate lymphocytic-plasmacytic enteritis	1	5
	Mild granulomatous inflammation with mild lacteal dilatation	1	4
Colon	Moderate neutrophilic colitis	1	4
	Severe neutrophilic and ulcerative colitis	1	9

*Scoring was conducted on the basis of WSAVA guidelines²²; scores ranged from 0 (no histologic change) to 9 (severe histologic changes).

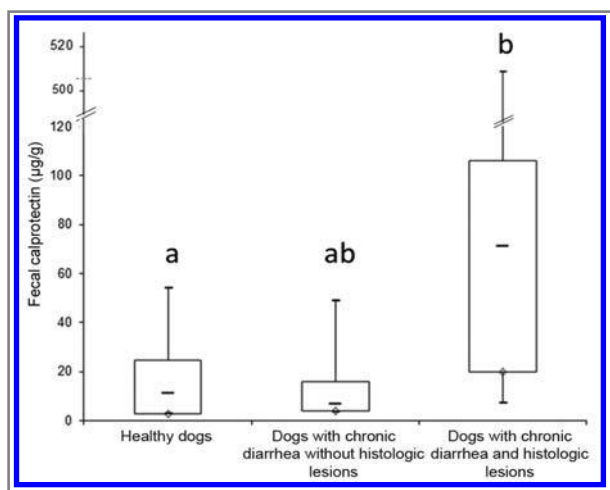


Figure 3—Box-and-whisker plot of fecal calprotectin concentrations in 69 healthy dogs and 14 dogs with chronic diarrhea (9 without histologic lesions and 5 with histologic lesions). See Figure 1 for key.

ity of 91.7% (95% CI, 62.1% to 100%), and area under the curve of 0.756 (95% CI, 0.579 to 0.933; $P = 0.005$; Figure 2).

Fecal calprotectin concentrations and histologic changes—A median of 6 adequate tissue biopsy specimens (range, 1 to 19) was obtained from the 27 dogs with chronic diarrhea. Histologic results were considered for 14 dogs on the basis of at least 6 adequate tissue samples; no interpretation of histologic lesions was possible for the remaining 13 dogs. Of the 14 dogs, differentiation of the location of the histologic lesion was not possible for 5 but was possible for 9 (location of the lesion was the duodenum in 7 dogs and the colon in 2 dogs). Gastroduodenoscopy associated with colonoscopy was performed in 6 dogs. Two of these 6 dogs did not have pathological histologic changes, whereas the other 4 dogs had histologic lesions in the duodenum ($n = 2$) or colon (2; Table 1). Gastroduodenoscopy alone was performed in 8 dogs, 5 of which had histologic lesions in the duodenum.

The fecal calprotectin concentration (median, 71.1 µg/g; range, 2.9 to 2,094.0 µg/g) in the 9 dogs with chronic diarrhea and histologic lesions was 10 times as high as the concentration (median, 6.9 µg/g; range, 3.5 to 71.1 µg/g) in the 5 dogs with chronic diarrhea but without histologic lesions; however, these concentrations did not differ significantly ($P = 0.083$). The fecal calprotectin concentration in dogs with chronic diarrhea and histologic lesions was significantly ($P = 0.006$) higher (6 times as high) than the concentration (median, 11.0 µg/g; range, 2.9 to 109.8 µg/g) in the 69 healthy control dogs (Figure 3). Fecal calprotectin concentrations were not significantly ($P = 0.711$) different between dogs with chronic diarrhea but without histologic lesions and healthy control dogs. Fecal calprotectin concentrations were significantly correlated ($r^2 = 0.23$; $P = 0.017$) with the severity of intestinal lesions. However, there was not a significant ($P = 0.199$) correlation between the severity of histologic lesions and the CCECAI.

Discussion

In the present study, fecal calprotectin concentrations were measured in healthy dogs and in dogs undergoing diagnostic evaluation because of chronic diarrhea. Fecal calprotectin concentrations were significantly higher in dogs with chronic diarrhea, compared with concentrations in healthy control dogs. These results suggested that calprotectin concentration could be a useful marker for noninvasive assessment of dogs with chronic diarrhea, as has been described for children and adult humans with IBDs (Crohn's disease and ulcerative colitis).^{8,9,15–17,25} A CCECAI ≥ 12 has been used as a cut-off value for the prediction of a negative outcome.⁵ In the present study, dogs with a CCECAI ≥ 12 had significantly higher fecal calprotectin concentrations than did dogs with a CCECAI < 12 . Therefore, fecal calprotectin may be an objective surrogate marker of CCECAI and fecal calprotectin concentrations may be used to predict a negative outcome. The optimal cutoff concentration of fecal calprotectin to differentiate between these 2 populations of dogs was determined via analysis of an ROC; that value was 48.9 µg/g and was associated with moderate sensitivity and high specificity. In human patients, a similar calprotectin cutoff value of 50 µg/g is used for the differentiation of patients with active mucosal inflammation (with high sensitivity and specificity) and to predict clinical relapse.^{11,14,18,26}

Other gastrointestinal disorders, such as viral and bacterial gastroenteritis or intestinal neoplasia, have been associated with increased fecal calprotectin concentrations in humans.²⁷ The same may apply for dogs, although this has not yet been determined. Therefore, measurement of fecal calprotectin concentrations alone likely will not replace a full diagnostic evaluation that includes signalment, patient history, and results of physical examination, clinicopathologic testing, diagnostic imaging, and histologic evaluation of intestinal biopsy specimens. Instead, it is expected that the measurement of fecal calprotectin concentrations would offer an additional variable for noninvasive monitoring of disease activity.

In the present study, significantly higher fecal calprotectin concentrations were detected in dogs with diarrhea and histologic lesions, compared with concentrations in dogs with chronic diarrhea but no histologic changes or in healthy control dogs. Furthermore, fecal calprotectin concentrations were significantly correlated with the severity of histologic intestinal lesions. These results suggested that fecal calprotectin may be a useful biomarker for the presence of histologic intestinal lesions in dogs, as has been reported in humans.^{9,15–17,28,29} However, the cellular expression and distribution pattern of calprotectin have not been reported in dogs, and the measurement of fecal calprotectin concentrations may yield inconsistent results in dogs with intestinal diseases that are not characterized by neutrophilic infiltrates (eg, celiac disease or diverticular disease in humans).^{29,30} In the present study, dogs had some infiltration with lymphocytes, plasma cells, macrophages, or a combination of these cells. Therefore, further studies are needed to assess fecal calprotectin

concentrations in relation to the type of inflammatory infiltrates (granulomatous or neutrophilic enteropathy, lymphoplasmacytic enteropathy, and eosinophilic enteritis).

Practitioners typically rely on clinical signs for evaluation of a patient's response to treatment because follow-up endoscopy to determine response to treatment is invasive and cannot be performed routinely. Moreover, histologic lesions are an unreliable indicator of treatment response in canine patients with chronic enteropathies.³¹ In that study,³¹ the total number of lymphocytes in the duodenal mucosa of dogs with IBD did not change after patients were treated successfully with prednisone and metronidazole. Moreover, no correlation was detected between the efficacy of treatment (as reflected by the canine IBD activity index score) and the severity of histologic lesions in that study.³¹ Therefore, fecal calprotectin concentrations may be an appropriate and objective marker for evaluation of the response to treatment in dogs with chronic enteropathies. In humans, fecal calprotectin concentrations decrease with successful treatment of ulcerative colitis and correspond with clinical, endoscopic, and histologic improvement.³² Prospective studies are needed to evaluate the role of fecal calprotectin concentrations in dogs in clinical settings.

Analysis of the results of the present study suggested that fecal calprotectin concentrations may be a useful marker of disease severity when used in combination with histologic evaluation of intestinal biopsy specimens. Further studies are needed to assess the clinical usefulness of measuring fecal calprotectin concentrations in dogs with various gastrointestinal diseases.

a. Tanagra, version 1.4.48, Ricco Rakotomalala, Lyon, France.

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