
Effect of intestinal inflammation on fecal elastase concentration in dogs

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Background: A commercially available ELISA kit for fecal elastase measurement can be used in the diagnosis of exocrine pancreatic insufficiency (EPI) in dogs. However, other causes of diarrhea also may affect fecal elastase concentration. **Objective:** This study was undertaken to determine whether intestinal inflammation alters fecal elastase concentration in dogs. **Methods:** Fecal elastase concentration was measured with an ELISA kit in the following groups of dogs: group 1 (n=16), control dogs, without gastrointestinal disease; group 2 (n=14), dogs with diarrhea and no histopathologic evidence of intestinal inflammation; and group 3 (n=12), dogs with diarrhea and histopathologic evidence of intestinal inflammation. Serum trypsin-like immunoreactivity (TLI) was determined in dogs with diarrhea to rule out EPI. **Results:** All dogs in groups 2 and 3 had serum TLI concentrations >5 µg/L, ruling out EPI. No statistically significant difference was found in fecal elastase concentration among the 3 groups of dogs (P=.969). **Conclusions:** The results indicate that intestinal inflammation does not affect fecal elastase concentration, such that test results may be used to exclude a diagnosis of EPI even in animals with inflammatory bowel disease. (*Vet Clin Pathol.* 2005;34:49-51)

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Exocrine pancreatic insufficiency (EPI) in dogs can occur as a consequence of a variety of pathologic processes, resulting in >90% loss of the secretory capabilities of the pancreas.¹ Clinical signs reported with EPI include profound weight loss, ravenous appetite, and soft or diarrheic stools, although the appearance of feces in some cases can be normal. These clinical signs, however, are not specific for EPI and can occur in animals with small intestinal malabsorption, such as that caused by idiopathic intestinal inflammation (inflammatory bowel disease). Thus, in clinical practice, a veterinarian may run a fecal elastase test to exclude EPI before proceeding with further investigations such as endoscopy and intestinal biopsy.

Other diagnostic tests, including assays of proteolytic activity in feces by gelatin and azocasein gel digestion, may be performed on feces to exclude a diagnosis of EPI in dogs with malabsorption, but these techniques are unreliable.² The fecal elastase test was developed as an improved diagnostic method for EPI. Of course, the serum canine trypsinlike immunoreactivity (cTLI) assay remains the "gold standard" for the laboratory diagnosis of EPI, with a reported sensitivity and specificity of 100%.³ However, the fecal elastase ELISA may have an advantage over serum cTLI in that the test is nonradioactive; hence, an isotopic laboratory is not required. In addition, it obviates the need of blood sample collection.

Following work confirming the stability of pancreatic elastase in the intestine of the dog, a canine-specific antibody was developed.⁴ Its use in an ELISA for measurement of canine pancreatic elastase 1 (E1) in feces was validated by Spillman.⁵ In that study, using single-day samples at a cut off level of 10 µg E1/g feces, sensitivity was 95.3% and specificity was 92.0% for

diagnosing EPI. However, no study has looked at fecal elastase concentrations in dogs with intestinal inflammation.

Elastase is a serine protease found not only in the pancreas but also in neutrophils. Pancreatic elastase is produced as a zymogen and, after activation within the intestinal lumen, digests elastin. The physiologic role of neutrophil elastase is to degrade foreign material ingested by phagocytosis. Furthermore, there is only moderate sequence homology between the 2 enzymes, resulting in separate enzyme classification (pancreatic elastase [EC 3.4.21.36] and neutrophil elastase [EC 3.4.21.37]).⁶ Although the possibility of cross-reaction by neutrophil elastase in the fecal ELISA is unlikely, further work to evaluate the potential for false-positive results in dogs with EPI and intestinal inflammation is required.

The purpose of the present study was to use a commercially available species-specific immunoassay (Elastase-1 Canine, ScheBo Biotech UK Limited, Basingstoke, UK) to determine whether intestinal inflammation alters fecal elastase concentration in dogs without EPI, thereby allowing a clinician who uses the test for ruling out EPI to be confident in the results.

Materials and Methods

Study animals

Control dogs (group 1) were presented to the Small Animal Hospital at the University of Liverpool for investigation of diseases that did not involve the gastrointestinal tract. Group 1 contained 10 males (2 neutered) and 6 females (1 neutered);

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mean age was 5.0 years (0.5–10 years), and 3 of the dogs were mixed breeds. Serum cTLI was not measured and intestinal biopsies were not obtained, so there was no evidence to exclude intestinal inflammation or EPI conclusively. However, none of the dogs had clinical evidence of gastrointestinal disease such that further investigations were not legally or ethically justifiable within UK legislative guidelines of animal care.

Samples also were collected from 26 dogs presented to the Small Animal Hospital at the University of Bristol for investigation of chronic diarrhea. In all dogs, serum TLI concentration was determined and endoscopic intestinal biopsies were performed as part of the clinical investigation of diarrhea. On the basis of the biopsy results (as described below) the dogs were subsequently categorized into those without intestinal inflammation (group 2) and those with intestinal inflammation (group 3). Group 2 contained 8 males (5 neutered) and 6 females (3 neutered); mean age was 4.3 years (1–13 years), and 1 of the 14 dogs was a mixed breed. Group 3 contained 7 males (3 neutered) and 5 females (3 neutered); mean age was 5.2 years (1–12 years), and 1 of the 12 dogs was a mixed breed.

Biopsy and histopathology

Dogs with diarrhea were prepared for endoscopy by withholding food for 18–24 hours. Gastroduodenoscopy was performed under general anesthesia with a GIF-XQ230 flexible video endoscope (Olympus Keymed, Southend-on Sea, UK). Multiple mucosal biopsies were taken at the level of the caudal duodenal flexure with FB-25K biopsy forceps (Olympus Keymed). Samples were placed in 10% neutral buffered formalin and routinely processed for histopathologic examination.

Histopathologic scoring was performed by a single histopathologist (MJD) after examination of H&E-stained sections of the intestinal biopsies collected from the dogs with diarrhea. Biopsies were graded as follows: grade 0, normal mucosal cellularity and architecture; grade 1, normal mucosal architecture but mild increases in cellularity of lamina propria or mild increases in the number of intraepithelial lymphocytes; grade 2, disruption of normal architecture with moderate increases in cellularity of the lamina propria; grade 3, villus stunting, ulceration, crypt hyperplasia, or crypt abscessation and marked increases in lamina propria or intraepithelial lymphocyte cellularity.

Dogs with no clear histologic evidence of intestinal inflammation were assigned to group 2 (histologic grades 0 and 1, $n=14$). Dogs with clear histologic evidence of intestinal inflammation were assigned to group 3 (histologic grades 2 and 3, $n=12$). Dogs with different histologic scores were combined because there were insufficient numbers of dogs with grades 0 and 3 to allow statistical examination of 4 separate histologic groups. Dogs in group 3 had either predominantly lymphocytic-plasmacytic ($n=9$) or eosinophilic ($n=3$) inflammation.

Fecal elastase determination

All fecal samples from dogs were collected within a 6-month period and were frozen at -20°C to allow samples to be batched and analyzed simultaneously. Pancreatic elastase has been reported to be stable for at least 1 year at this

temperature. Extraction of fecal E1 was performed with E1 Canine Quick Prep canine tubes (ScheBo Biotech UK Limited) as per the manufacturer's instructions. Fecal samples were excluded if very high water content did not allow accurate stool sampling with the Quick Prep canine tubes, to ensure accurate stool sample weight and to reduce a dilutional effect on fecal elastase concentration.

Fecal E1 was measured with the Elastase-1 Canine ELISA kit as per the manufacturer's instructions.⁷ The manufacturer's reference concentrations are $<10\ \mu\text{g E1/g}$ stool for animals with severe EPI and $>40\ \mu\text{g E1/g}$ stool for animals without EPI. Retesting is recommended if concentrations are between these values. For the purpose of this study, we quantified elastase concentrations after serial dilutions of fecal samples, so any significant difference between controls and dogs with intestinal disease but without EPI (ie, E1 levels $>40\ \mu\text{g/g}$) could be determined.

Doubling dilutions of each fecal sample were made from a starting dilution of 1:40, and all samples were fully titrated together to a final dilution of 1:5120 with the standard on each plate. The most concentrated standard ($180\ \mu\text{g E1/g}$ stool) from the ELISA kit was used as the starting point for the dilution series of the standard. A series of negative controls was included, and 1 component of the ELISA was omitted from each of these. A Multiskan Biochromatic plate reader (Labsystems, Helsinki, Finland) was used to measure the optical density at 450 and 620 nm. All samples and standard curves were assessed for parallelism by PROBIT analysis.⁸ Sample curves that were not parallel to the standard were excluded. The value of each sample was calculated from a minimum of 3 dilution points.

Statistical analysis

Statistical analysis was done using Minitab Release 13 (Minitab Inc, Coventry, UK). The results were assessed for normality both before and after logarithmic transformation with a Kolmogorov-Smirnov test. The results were not normally distributed before or after transformation; therefore, comparisons among groups were made by using nonparametric tests. A Kruskal-Wallis test was used to compare fecal elastase concentration based on sex and study group. Spearman correlation coefficient was used to examine the correlation between fecal elastase concentration and age. A value of $P < .05$ was considered significant for all analysis.

Results and Discussion

All dogs in groups 2 and 3 had serum TLI concentrations $>5\ \mu\text{g/L}$, excluding EPI as the cause of diarrhea. No statistically significant difference in fecal elastase concentration was found between control dogs (group 1, median $12,764\ \mu\text{g/g}$, range $346\text{--}12,004\ \mu\text{g/g}$), dogs without intestinal inflammation (group 2, median $11,538\ \mu\text{g/g}$, range $672\text{--}100,597\ \mu\text{g/g}$) and dogs with intestinal inflammation (group 3, median $10,016\ \mu\text{g/g}$, range $362\text{--}35,207\ \mu\text{g/g}$) ($P=.969$) (Figure 1). There was no significant effect of age ($R^2=.307$, $P=.06$) or sex ($P=.143$) on fecal elastase concentration in all samples combined.

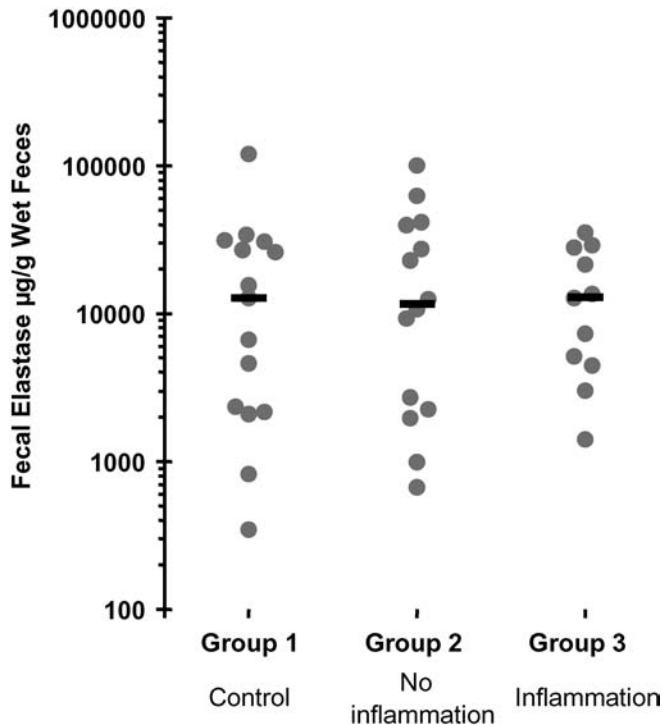


Figure 1. Fecal elastase concentrations ($\mu\text{g/g}$ of wet feces) in control dogs without gastrointestinal disease (group 1), dogs with diarrhea but without intestinal inflammation (group 2), and dogs with diarrhea and intestinal inflammation (group 3). Solid bars represent mean values for each group. No significant difference was found among the 3 groups ($P = .969$). In groups 1 and 3, a number of dogs had the same elastase value, resulting in superimposition of the circles; hence, the number of circles does not match the number of dogs in each group.

The results of this study demonstrated that the concentration of fecal elastase was not affected by intestinal inflammation in dogs. Interestingly, the fecal concentrations of E1 in all groups of dogs in this study were substantially higher than those previously reported (0–3952.0 $\mu\text{g/g}$).⁹ We were unable to explain this difference, although it may be

a consequence of the dilution protocol or different laboratory conditions. The higher values do not affect interpretation, as all fecal elastase values were above the reference range.

In light of these results, it may be concluded that intestinal inflammation has no effect on fecal elastase concentration, as measured using the Elastase-1 ELISA kit, such that test results may be used to exclude a diagnosis of EPI even in animals with inflammatory bowel disease.

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