Canine faecal pancreatic elastase (cE1) in dogs with clinical exocrine pancreatic insufficiency, normal dogs and dogs with chronic enteropathies

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Abstract

Faecal canine pancreatic elastase (cE1) concentration was measured in faeces samples of dogs with clinical exocrine pancreatic insufficiency (EPI), normal dogs of different breeds, normal Beagles, German Shepherd Dogs (GSD), Rough Coated Collies (RCC) and dogs with chronic enteropathies. Using single day faeces samples median faecal cE1 concentration was 1.6 µg/g (0–18.0) in dogs with clinical EPI, 253.2 µg/g (0–3952.0) in normal dogs, 618.2 µg/g (6.6-2929.0) in Beagles, 49.0 µg/g (0-567.0) in GSD/RCC and 360.0 µg/g (0–3472.0) in dogs with chronic enteropathies. Using faeces samples from three consecutive days median faecal cE1 values were 1.3 µg/g (0–10.8) in dogs with clinical EPI, 410.3 µg/g (111.0–1721.0) in Beagles, 49.0 µg/g (1.9–567.0 µg/g) in GSD/RCC and 262.0 µg/g (30.0–1694.0 µg/g) in dogs with enteropathies. It is concluded that faecal cE1 determination can be used to diagnose or exclude clinical EPI in dogs with chronic diarrhoea.

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Introduction

Pancreatic Elastase 1 (E1) is produced in the acinar cells of the exocrine pancreas. Studies in humans have shown that E1 is highly stable during the intestinal passage because it is not degraded by endogenous or bacterial proteases and its faecal concentration correlates directly with the pancreatic enzyme output. Human faecal E1 is easily detected by a species specific ELISA and its determination is of increasing importance in human medicine as a non invasive pancreatic function test with a sensitivity and specificity of 93% each. The E1 test results correlate significantly with the secretin pancreozymin test as the golden standard for the assessment of exocrine pancreatic function in humans (Lipps 1996; Löser and Fölsch 1996, Löser and others 1996).

Recently a study in dogs revealed that canine faecal E1 could be detected with polyclonal anti-human pancreatic E1 antibodies by rocket immunoelectrophoresis. It was shown that the concentration of faecal cE1 determined by rocket immunoelectrophoresis is highly sensitive for EPI in dogs with a moderate specificity of this method due to the lowest detection limit of 200 µg/g. To improve the methodology by decreasing the lower detection limit a species specific ELISA for cE1 had to be developed (Spillmann and others 1998a). With the develop-

ment of monoclonal anti-canine pancreatic E1 antibodies it became possible to determine cE1 concentrations with a species specific immunological test system (Eim, 1998). To assess the diagnostic value of the ELISA a study was performed to investigate the enzyme concentration in faeces samples of dogs with clinical EPI, normal dogs of different breeds and dogs with chronic enteropathies.

Materials and Methods

Dogs – 43 dogs with clinical EPI; 288 apparently healthy dogs of 98 different pure breeds; 23 normal Beagle dogs; 24 normal German Shepherd dogs (GSD); three normal Rough-coated Collies (RCC) and 39 dogs with chronic enteropathies were included in this study.

Study design – Single day faeces samples for faecal cE1 measurements were taken from 43 dogs with clinical EPI (cTLI < 2.5 µg/l), 288 dogs without a history of chronic or recurrent gastrointestinal problems and 39 dogs with chronic enteropathies (cTLI > 5.0 µg/l). Additionally faecal cE1 concentration was determined on three consecutive days in faeces samples from 39 dogs with clinical EPI, 23 normal Beagles, 24 normal GSD, three normal RCC (cTLI > 5.0 µg/l) and 8 dogs with chronic enteropathies but serum cTLI values within the reference range. All samples were stored at –20°C until measurement of faecal cE1.

Serum cTLI assay – Serum cTLI was assayed with the species-specific radioimmunoassay (cTLI RIA, Diagnostic Products Corporation, Los Angeles, CA) in a commercial laboratory (Williams and Batt, 1983).

Canine pancreatic elastase assay – Canine E1 concentration was determined with the species specific ELISA Elastase 1 – Canine (ScheBo•Biotech AG, Giessen, Germany) according to the manufacturers instructions.

Statistical analysis – The data of cE1 determination are expressed as median, quartiles, minimum and maximum because they were not normally distributed. The medians of cE1 concentrations of three consecutive days are calculated in every group by using the individual medians of the patients at three days. Non parametric statistical assessment of group differences in faecal cE1 concentration between dogs with clinical EPI, normal dogs of different breeds, Beagle dogs, GSD/RCC and dogs with chronic enteropathies was carried out for single day and three consecutive day results by the Kruskal-Wallis-test followed by multiple comparisons with Nemenyi test with the statistical software package BMDP (Dixon 1993). There was always used a significance value of $\alpha = 0.05$.

The diagnostic value of cE1 determination on single or three consecutive days is expressed as sensitivity and specificity as well as the positive and negative predictive value for clinical EPI in comparison to apparently normal dogs, normal Beagles, normal GSD/RCC and dogs with enteropathies.

Results

Using *single day faeces samples* for cE1 determination 43 *dogs with EPI* (cTLI < 2.5 µg/l) had median faecal cE1 concentration of 1.6 µg/g (0–18.0). In 288 *normal dogs of different breeds* median faecal cE1 value was 253.2 µg/g (0–3952.0). The difference between both groups was significant ($p < 0.0001$, Fig. 1). Median faecal cE1 values in single day samples of *normal Beagles and GSD/RCC* (cTLI > 5.0 µg/l) were 618.2 µg/g (6.6-2929) in Beagles and 49.0 µg/g (0-567.0) in GSD/RCC. In this groups median faecal cE1 concentration was calculated with cE1 values of the first day for group comparison with dogs with clinical EPI and normal dogs of other breeds. The group comparison with the Kruskal-Wallis-test showed statistical significant differences with $p < 0.0001$. Subsequent multiple comparisons by Nemenyi test revealed a significant difference in median cE1 concentration between dogs with clinical EPI and normal Beagles ($p < 0.0001$) as well as normal GSD/RCC ($p = 0.003$, Fig. 1). There were no significant differences in median cE1 values between Beagles and dogs of different breeds ($p = 0.343$). GSD/RCC had significantly lower median cE1 values than dogs of different breeds ($p = 0.049$) and Beagles ($p = 0.006$; Fig. 1). In 39 *dogs with chronic enteropathies* (cTLI > 5.0 µg/l) median faecal cE1 concentration was 360.0 µg/g (0–3472.0) in single day samples. The results were statistically higher than those of dogs with clinical EPI ($p < 0.0001$, Fig. 1). There was no statistical difference of this group with cE1 values of normal dogs of different breeds ($p = 0.84$), Beagle dogs ($p = 0.92$) but with cE1 values of normal GSD/RCC ($p = 0.029$, Fig. 1).

Using *faeces samples from three consecutive days* median cE1 value in 39 *dogs with clinical EPI* was 1.3 µg/g (0–10.8), in 23 *normal Beagles* 410.3 µg/g (111.00–1721.0) and in 27 *GSD/RCC* 49.0 µg/g (1.9–567.0). Faecal cE1 results of Beagles and GSD/RCC were grouped together for the statistical comparison with cE1 values of dogs with clinical EPI. The Nemenyi test revealed significantly lower cE1 values in EPI dogs than in normal Beagles together with GSD and RCC ($p < 0.0001$, Fig. 2). The difference between cE1 values of normal Beagles and GSD/RCC was also significant ($p < 0.0001$, Fig. 2).

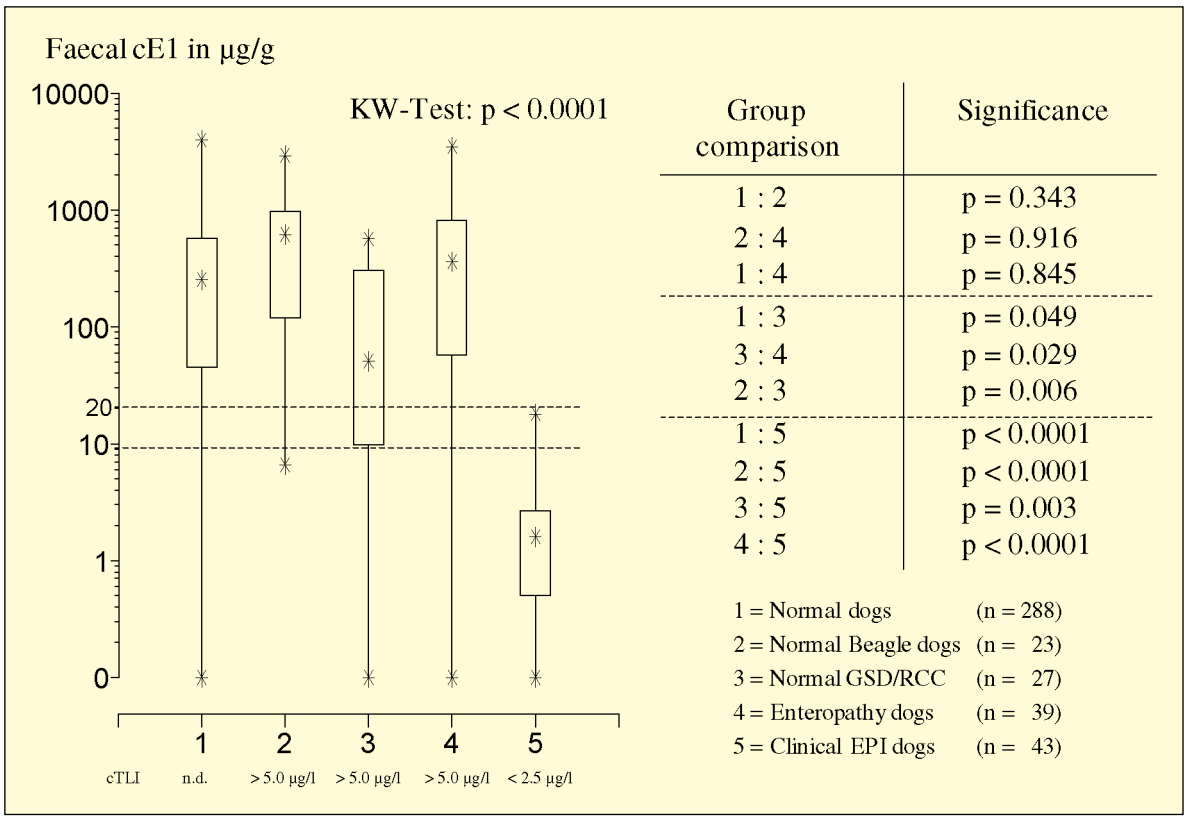

Figure 1 - Box-and-Whisker-Plot for the median concentration of faecal canine pancreatic elastase (cE1) in single day faeces samples and results of the statistical analysis with Kruskal-Wallis-test (KW-test) followed by group comparison in pairs after Nemenji.

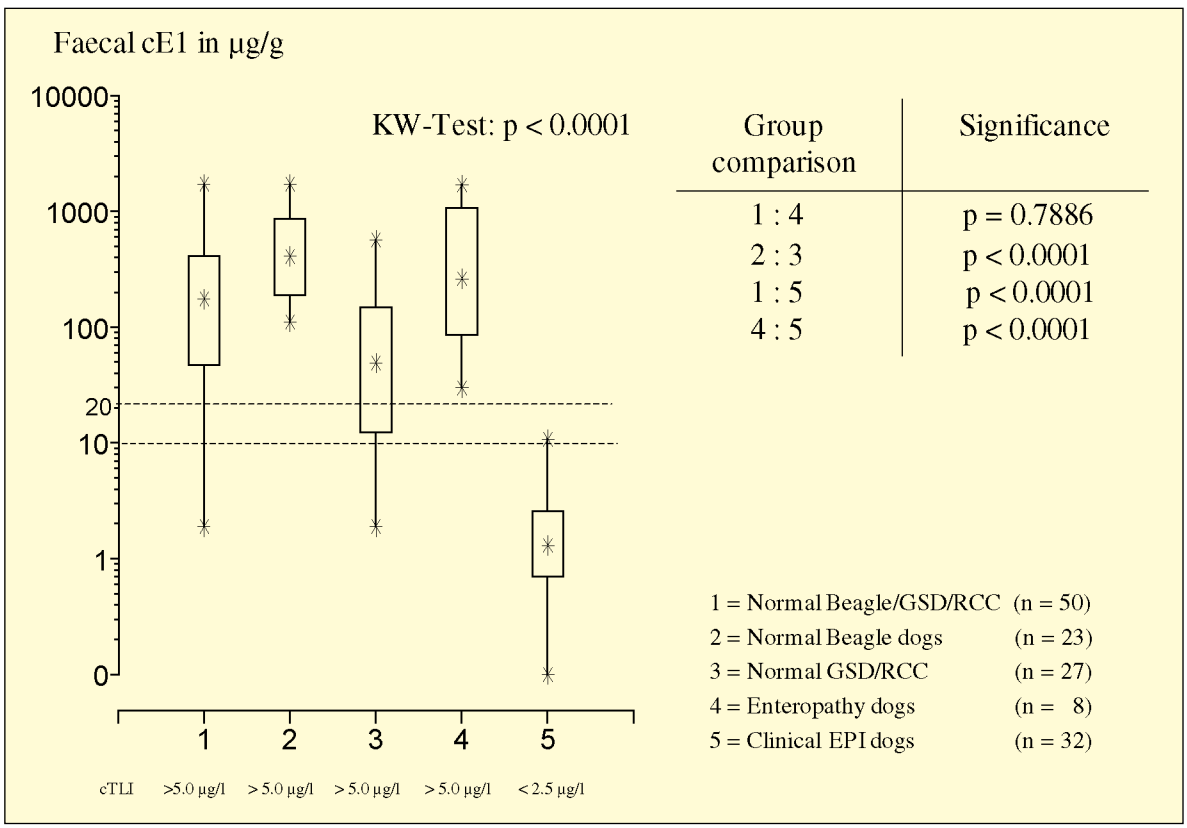

Figure 2 - Box-and-Whisker-Plot for the median concentration of faecal canine pancreatic elastase (cE1) in faeces samples from three consecutive days and results of the statistical analysis with Kruskal-Wallis-test (KW-test) followed by group comparison in pairs after Nemenji.

Eight dogs with chronic enteropathies observed for three consecutive days had median cE1 values of 262.0 µg/g (30.0–1694.0) in faeces samples. Statistical comparison with the results of normal Beagles grouped together with normal GSD/RCC revealed no significant differences ($p = 0.79$, Fig. 2). But the median cE1 value of dogs with clinical EPI was significantly lower than of dogs with chronic enteropathies ($p < 0.0001$, Fig. 2).

The *diagnostic value of faecal cE1 measurement* for the diagnosis of EPI within this study was evaluated by sensitivity and specificity as well as positive and negative predictive values at two cE1 cut off values (10.0 and 20.0 µg/g).

Sensitivity for clinical EPI at a cE1 cut off value of 10.0 µg/g was 95.3% with single day cE1 results and 96.9% with median cE1 values of three consecutive days. It was 100% for all compared groups at a cE1 cut off value of 20.0 µg/g.

Specificity was 92.0% at a cut off value of 10.0 µg/g and 85.5% at 20.0 µg/g comparing EPI dogs with normal dogs of different breeds. The comparison of single day cE1 values of dogs with clinical EPI with cE1 results of normal Beagles showed a specificity of 91.3% at both cut off values. Specificity was 100% using median cE1 results of three consecutive days. Specificity estimated between dogs with clinical EPI and normal GSD/RCC revealed 70.4% for single day cE1 values or 77.8% for median cE1 values of three days at a cut off value of 10.0 µg/g. It was 62.0% (single day) or 59.3% (three days) at a cut of value of 20.0 µg/g (Tab. 1). Comparison of cE1 values of EPI dogs with cE1 results of dogs with chronic enteropathies revealed a specificity of 92.3% (cut off = 10.0 µg/g) or 89.7% (cut off = 20.0 µg/g).

In this study at a cut off value of 10.0 (20.0) µg/g the *positive predictive value* for clinical EPI was 64.4% (50.6%) comparing dogs with clinical EPI with normal dogs but 95.3% (95.5%) in comparison with normal Beagles, 83.7% (81.0%) with normal GSD/RCC and 93.2% (91.5%) with enteropathy dogs. Using median cE1 values of three consecutive days in Beagles and GSD/RCC positive predictive value was 100% or 83.8% (74.4%). The *negative predictive value* was over 90.0% in all groups with a cut off value of 10.0 µg/g but 100% in all group comparisons with a cut off value of 20.0 µg/g (Tab. 2).

Discussion

The present study was designed to evaluate the diagnostic value of canine faecal pancreatic elastase (cE1) for clinical EPI in comparison with normal dogs and patients with primary chronic enteropathies.

At present determination of canine trypsin like immunorectivity (cTLI) in serum with a species specific radioimmunoassay (RIA) is the recommended diagnostic tool for the diagnosis of EPI in dogs. In accordance to literature serum cTLI concentrations < 2.5 µg/g were used to diagnose and cTLI values > 5.0 µg/g to exclude clinical EPI within this study (Williams and Batt, 1983, Williams and Batt, 1988; Simpson and others 1991, Spillmann and others 1994, Williams, 1995; Willard 1998, Wiberg and others 1999a).

Pancreatic elastase (E1) is produced by the exocrine pancreas only and is secreted into the intestine. Faecal E1 concentration reflects directly the exocrine pancreatic function in humans with a sensitivity and specificity of 93% each for clinical EPI (Lipps 1996; Löser and Fölsch 1996, Löser and others 1996). It is known that human E1 is stable during intestinal passage with increasing concentration from pancreatic juice to stool (Sziegoleit and others, 1989). The same phenomenon was seen in a study comparing canine E1 concentration in chyme of different intestinal parts of dogs. The cE1 concentration in faeces samples out of the rectum was much higher than in chyme samples from duodenum (Spillmann and others 1998b). Furthermore the investigators could prove that the immunological determination of cE1 in faeces is not influenced by enzyme supplementation and the Elastase 1 - Canine test does not cross react with elastase from other species.

The recent study revealed that dogs with clinical EPI and serum cTLI values < 2.5 µg/l have significantly lower faecal cE1 values than normal dogs of different breeds, normal Beagles or GSD/RCC as well as dogs with chronic enteropathies in single day faeces samples or samples from three consecutive days. Consistently low faecal cE1 concentrations < 10.0 µg/g were found in faeces samples of three consecutive days in 96.9% of these dogs and 100% of them had cE1 values < 20.0 µg/g. Thus the sensitivity of cE1 results for EPI is 100% at a cut off value of 20.0 µg/g which is equal to the sensitivity of cTLI determination (Williams an Batt 1988). This high sensitivity was found comparing the cE1 results of EPI dogs with the cE1 values of all normal dog groups and of dogs with chronic enteropathies regardless whether faeces samples were taken on a single day or on three consecutive days.

To investigate faecal cE1 values in normal dogs faeces samples were collected at a breed exhibition from 288 pure breed dogs with no history of chronic or recurrent gastrointestinal signs. The determination of faecal cE1 concentration in single day faeces samples revealed that faecal cE1 values < 10.0 µg/g could be found in 8.0% and < 20.0 µg/g in 14.5% of them. However, the negative predictive value of

Table 1 - Sensitivity and specificity of faecal cE1 values of single day faeces samples or median faecal cE1 values from three consecutive days comparing apparently normal dogs, normal Beagles and normal GSD / RCC (cTLI > 5.0 µg / g) with dogs with clinical EPI (cTLI < 2.5 µg / g)

Group comparison Dogs with clinical EPI* (n = 43 / 32) ** versus	Sensitivity and Specificity for clinical EPI in %							
	cE1 cut off = 10.0 µg / g				cE1 cut off = 20.0 µg / g			
	Sensitivity		Specificity		Sensitivity		Specificity	
	Single day	Three days	Single day	Three days	Single day	Three days	Single day	Three days
Normal dogs (n = 288)	95.3	n. d.	92.0	n. d.	100	n. d.	85.5	n. d.
Normal Beagles* (n = 23)	95.3	96.8	91.3	100	100	100	91.3	100
Normal GSD / RCC* (n = 27)	95.3	96.8	70.4	77.8	100	100	62.0	59.3
Dogs with enteropathies (n = 39)	95.3	n. d.	92.3	n. d.	100	n. d.	89.7	n. d.

* Reference test = serum c TLI; ** (n = single day / three days); n.d. = not done.

Table 2 - Positive and negative predictive values of faecal cE1 values of single day faeces samples or median faecal cE1 values from three consecutive days comparing apparently normal dogs, normal Beagles and normal GSD / RCC (cTLI > 5.0 µg / g) with dogs with clinical EPI (cTLI < 2.5 µg / g)

Group comparison Dogs with clinical EPI* (n = 43 / 32) ** versus	Predictive value for clinical EPI in %							
	cE1 cut off = 10.0 µg / g				cE1 cut off = 20.0 µg / g			
	Positive		Negative		Positive		Negative	
	Single day	Three days	Single day	Three days	Single day	Three days	Single day	Three days
Normal dogs (n = 288)	64.4	n. d.	99.2	n. d.	50.6	n. d.	100	n. d.
Normal Beagles* (n = 23)	95.3	100	91.3	95.8	95.5	100	100	100
Normal GSD / RCC* (n = 27)	83.7	83.8	90.5	95.4	81.0	74.4	100	100
Dogs with enteropathies (n = 39)	93.2	n. d.	94.7	n. d.	91.5	n. d.	100	n. d.

* Reference test = serum c TLI; ** (n = single day / three days); n. d. = not done.

99.2% with the lower or 100% with the higher cut off value has to be assessed as very good for exclusion of clinical EPI. To understand the reasons for occasionally low cE1 values in apparently normal dogs further studies should to be carried out to investigate the influence of breed, food, feeding time and subclinical EPI on faecal cE1 concentration.

To assess possible breed influences on faecal cE1 concentration normal GSD/RCC and Beagles were included in this study. It was seen that normal GSD/RCC have significantly lower faecal cE1 values than normal Beagles or normal dogs of different breeds. This result is very interesting since GSD and RCC are known as breeds with a high prevalence of EPI caused by hereditary pancreatic acinar atrophy (Von Weber and Freudiger, 1977, Westermarck, 1980, Westermarck et al. 1989, Wiberg and others 1999a/b). Thus, the lower specificity and positive predictive values found by comparing the cE1 results of GSD/RCC with the results of dogs with clinical EPI might be connected to this genetic predisposition. However, there were no differences in sensitivity (100%) and negative predictive value (100%) between GSD/RCC and Beagles at a cut off value of 20.0 µg/g (Tabb. 1 and 2).

The diagnostic value of faecal cE1 determination is clinically relevant for the confirmation or exclusion of clinical EPI in dogs with chronic diarrhoea and weight loss. Therefore faecal cE1 was determined in dogs with chronic diarrhoea due to primary diseases of the small intestine and compared with

the results of dogs with clinical EPI and cE1 values of normal dogs of different breeds, normal Beagles and GSD/RCC. The faecal cE1 concentration was significantly higher in dogs with chronic enteropathies than in dogs with clinical EPI and there were no median cE1 values < 20 µg/g in faeces of dogs with chronic enteropathies when faecal samples were taken on three consecutive days. Furthermore there were no statistically significant differences between dogs with chronic enteropathies and normal dogs of different breeds and Beagles. Thus faecal cE1 determination can be used to differentiate between dogs with chronic enteropathies and dogs with clinical EPI with high sensitivity, specificity and positive or negative predictive value even using single day faeces samples (Tabb. 1 and 2).

In summary the study revealed a high negative predictive value (100%) of cE1 determination for clinical EPI which can be excluded when cE1 concentration is > 20.0 µg/g. Although low cE1 values can occasionally be found in faeces samples of normal dogs faecal cE1 values < 20.0 µg/g or even < 10.0 µg/g together with typical clinical symptoms are suggestive for clinical EPI. The disease can be proven by repetition of faecal cE1 determination since dogs with clinical EPI will have consistently low cE1 values < 20.0 µg/g in contrast to normal Beagles, GSD/RCC or dogs with chronic enteropathies.

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