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Agreement of Serum Spec cPL with the 1,2-o-Dilauryl-Rac-Glycero Glutaric Acid-(6′-methylresorufin) Ester (DGGR) Lipase Assay and with Pancreatic Ultrasonography in Dogs with Suspected Pancreatitis

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Background: Spec cPL is the most sensitive and specific test for diagnosing pancreatitis in dogs. Its results have not been compared to those of the 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester (DGGR) lipase assay or those of abdominal ultrasonography.

Objectives: To investigate agreement of Spec cPL with DGGR lipase activity and pancreatic ultrasonography in dogs with suspected pancreatitis.

Animals: One hundred and forty-two dogs.

Methods: DGGR lipase activity (reference range, 24–108 U/L) and Spec cPL were measured using the same sample. The time interval between ultrasonography and lipase determinations was <24 hours. The agreement of the 2 lipase assays at different cutoffs and the agreement between pancreatic ultrasonography and the 2 tests were assessed using Cohen's kappa coefficient (κ).

Results: DGGR lipase (>108, >216 U/L) and Spec cPL (>200 μg/L) had κ values of 0.79 (95% confidence interval [CI], 0.69–0.9) and 0.70 (CI, 0.58–0.82). DGGR lipase (>108, >216 U/L) and Spec cPL (>400 μg/L) had κ values of 0.55 (CI, 0.43–0.67) and κ of 0.80 (CI, 0.71–0.9). An ultrasonographic diagnosis of pancreatitis and DGGR lipase (>108, >216 U/L) had κ values of 0.29 (CI, 0.14–0.44) and 0.35 (CI, 0.18–0.52). Ultrasonographically diagnosed pancreatitis and Spec cPL (>200, >400 μg/L) had κ values of 0.25 (CI, 0.08–0.41) and 0.27 (CI, 0.09–0.45).

Conclusions and Clinical Importance: Although both lipase assays showed high agreement, agreement between ultrasonography and lipase assays results was only fair. Because lipase results are deemed more accurate, ultrasonography results should be interpreted carefully.

Key words: Diagnostic; Imaging; Laboratory; Pancreas.

Pancreatitis is a relatively common disorder in dogs, and its diagnosis is clinically challenging. Depending on disease severity, clinical presentation can vary markedly and may consist of nonspecific findings such as anorexia, vomiting, lethargy, diarrhea, abdominal pain, and weight loss.[1–3] However, this combination of clinical signs can occur in other conditions. Controversy exists regarding the sensitivity and specificity of diagnostic tests for the diagnosis of pancreatitis. Part of this confusion arises from the fact that there is no easily applied gold standard against which diagnostic methods can be evaluated. A definitive diagnosis of pancreatitis requires histopathologic confirmation, but because of the invasiveness of pancreatic biopsy, and the possibility of highly localized disease that can be missed with a single biopsy,[4] this procedure is performed infrequently. Thus, the diagnosis of pancreatitis generally is clinical and based on a combination of clinicopathologic and imaging findings. Considering laboratory work, the Spec cPL currently is regarded as the most sensitive and specific test for diagnosing pancreatitis[5–7] and usually is accepted as a biochemical surrogate marker for the disease in clinical practice.[8,9] At present, it is widely believed that catalytic assays for measuring serum lipase activity are unreliable because of unsatisfactory sensitivity and specificity.[9–13] Care must be taken when interpreting results, however, because different assays for determination of serum lipase have been used, and the majority of studies refer to a method using 1,2-diglyceride (1,2DiG) as the substrate.[1,5,6,12] In 2001, a novel catalytic assay, the 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester (DGGR) assay, for colorimetric determination of serum lipase activity was introduced. Graca et al validated the DGGR lipase assay for use in dogs, and initial results in dogs with a clinical and ultrasonographic diagnosis of pancreatitis showed high sensitivity and moderate specificity.[14] This assay has not been investigated further in the dog. The DGGR lipase assay was incorporated into the serum biochemistry panel at our institution in 2005, we believe it has been useful in the investigation of pancreatitis, as recently shown in cats.[15]

Abbreviations:

CI	confidence interval
CV	coefficient of variation
DGGR	1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester
κ	Cohen's kappa coefficient

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In addition to serum lipase assays, pancreatic ultrasonography also has been used in the diagnosis of pancreatitis. Changes in pancreatic size, alterations in pancreatic and mesenteric echogenicity as well as detection of focal lesions generally are regarded as useful ultrasonographic features for the diagnosis of pancreatitis in dogs.[16–19] Although ultrasonography is regarded as a very sensitive diagnostic modality when performed by board-certified radiologists, the best reported sensitivity only reached 68%.[1] Static images from dogs with fatal acute pancreatitis were reviewed in that study, not necessarily reflecting the more variable presentation seen in clinical practice. Although pancreatic ultrasonography is routinely performed in specialty practice and with increasing frequency in general practice, newer studies using modern equipment and comparing findings with the most commonly used laboratory assays for pancreatitis are lacking. Therefore, the aim of this study was 2-fold: first, to evaluate the agreement of results of the Spec cPL and DGGR lipase methods in dogs with suspected pancreatitis, and second to evaluate the agreement of pancreatic ultrasonography results with the results of both lipase assays. On the basis of empirical clinical data, we hypothesized that the 2 serum lipase assays would show high agreement, whereas the agreement of pancreatic ultrasonography with results of lipase tests was expected to be only fair.

Material and Methods

Analytical Performance of the DGGR Lipase Assay

Because the DGGR lipase assay had been validated previously in dogs by Graca et al,[14] validation in this study consisted of testing precision and linearity on canine serum samples.

Precision

For within-run precision, canine serum samples were collected and pooled according to DGGR lipase activity as low (<13.7 U/L), medium (40 U/L), and high (>1,370.4 U/L). Fifteen tests were performed consecutively from each of the 3 canine serum pools. Day-to-day precision was measured with residual serum samples from 2 dogs with high and normal lipase activity. Both were analyzed once daily before analyzing patient samples during a 21-day period. From the results, random error was determined by calculation of the coefficient of variation (CV).

Linearity

Linearity of the measurement range was assessed for canine serum. To obtain values below and above the reference intervals, 1 canine sample with high DGGR lipase activity (2,807 U/L) was diluted with 0.9% saline solution in steps of 10% to obtain a dilution series from 0 to 100% (undiluted). The diluted and undiluted serum samples were analyzed in duplicate.[20]

Animals and Study Design

From November 2009 through March 2013, dogs with suspected pancreatitis were included. Pancreatitis was suspected when at least 2 of the following clinical signs were present: vomiting, anorexia, abdominal pain, or lethargy. The following variables were included and evaluated: signalment, serum DGGR lipase activity, Spec cPL concentration, serum albumin concentration, and results of pancreatic ultrasonography. Only dogs that had both lipase assays performed using the same blood sample were considered eligible for inclusion in the study. Repeated measurements in the same dogs were excluded. All serum samples were processed immediately after collection. DGGR lipase activity was measured using an in-house assay.[a] The reference range for DGGR lipase (24–108 U/L) had been previously established using 75 apparently healthy dogs of various breeds and either sex. Spec cPL was measured by IDEXX Laboratories.[b] Results of pancreatic ultrasonographic studies only were included when performed by a board-certified radiologist or by a radiology resident under direct supervision of a board-certified radiologist. The ultrasonographer was blinded to the lipase results. Ultrasonographic data also were only included if the time interval between pancreatic ultrasonography and serum lipase determinations was ≤24 hours. Ultrasonographic studies were performed using state-of-the-art equipment[c] and recorded as 2-D images. The following ultrasonographic variables were collected from the ultrasonography reports: radiologic pancreatic diagnosis (pancreatitis, yes or no), pancreatic enlargement, pancreatic echogenicity (hypoechoic, mixed-echoic, hyperechoic), surrounding mesenteric hyperechogenicity, and peritoneal fluid. Only dogs with complete ultrasonographic studies were included; all variables noted above must have been specifically mentioned (ie, present or absent) in the report. Results from pancreatic histopathology were included for comparison with lipase results, if the time interval between histopathology and serum lipase determination was ≤7 days.

Statistical Analysis

Statistical analysis was performed using commercial software.[d] Agreement between DGGR lipase and Spec cPL at various cutoffs was assessed using Cohen's kappa coefficient (κ).[21] Values between 0 and 0.20 indicated slight agreement, values between 0.21 and 0.40 indicated fair agreement, values between 0 .41–0.60 indicated moderate agreement, values between 0.61 and 0.80 indicated substantial agreement, and values between 0.81 and 1 indicated almost perfect agreement.[22] Agreement of pancreatic ultrasonography (diagnosis and variables) with DGGR lipase and with Spec cPL also was assessed using Cohen's kappa coefficient (κ). A Spearman correlation coefficient between results of the 2 lipase methods was also calculated.

Results

Analytical Performance of the DGGR Lipase Assay

Results from the precision study are shown in Table 1. CVs ranged from 0.8 to 3.6%, indicating that the DGGR lipase assay showed high precision at all ranges. The DGGR lipase assay demonstrated excellent linearity (Fig 1).

Study Population

The study population consisted of 142 dogs including 72 male and 70 female dogs. Ages ranged from 0.5 to 16 years (median, 8 years). Weights ranged from 1.6 to 66 kg (median, 15.9 kg). Breeds included mixed breed dogs (n = 28), Jack Russell Terrier (n = 8), Labrador Retriever (n = 6), Yorkshire Terrier (n = 6), Rottweiler (n = 5), Boxer (n = 5), Chihuahua

Table 1. Day-to-day and within-run precision of the DGGR lipase assay for canine serum.

	Within-Run Precision (n = 15)		Day-to-Day Precision (n = 21)	
Lipase Activity	Mean ± SD (U/L)	CV (%)	Mean ± SD (U/L)	CV (%)
Low	13.7 ± 0.5	3.6	Not determined	Not determined
Middle	40 ± 0.5	1.3	52 ± 1	2.5
High	1,370.4 ± 10.3	0.8	127 ± 4	3.3

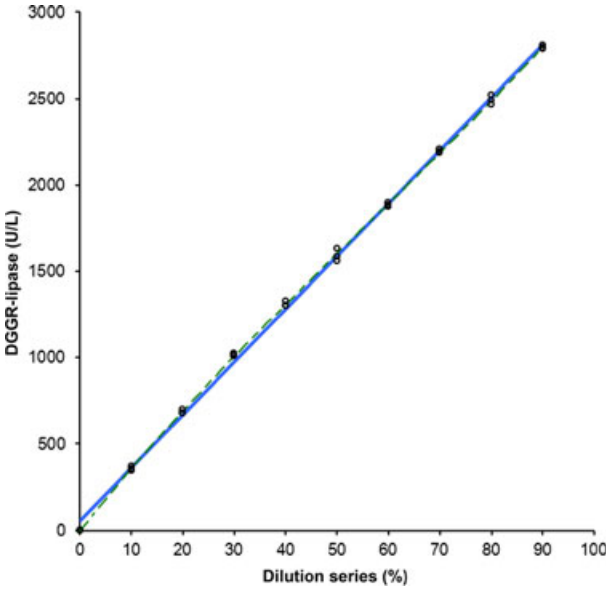

Fig 1. Linearity plot for lipase activity measured with the DGGR lipase assay (linear fit: $55.39 + 30.62x$; polynomial fit: $0.5767 + 37.07x - 0.1436x^2 + 0.0008589x^3$).

(n = 4), Cocker Spaniel (n = 4), German Shepherd (n = 3), Bernese Mountain Dog (n = 3), King Charles Cavalier Spaniel (n = 3), and other breeds.

DGGR lipase was ≤108 U/L in 42/142 (29.6%) dogs, between 108 and 216 U/L in 26/142 (18.3%) dogs, and >216 U/L in 74/142 (52.1%) dogs. Spec cPL was ≤200 μg/L in 55/142 (38.7%) dogs, between 201 and 399 μg/L in 18/142 (12.7%) dogs, and ≥400 in 69/142 (48.6%) dogs.

Agreement between DGGR Lipase and Spec cPL

When a DGGR lipase cutoff >108 U/L (above the reference range) and a Spec cPL cutoff >200 μg/L (increased concentration suggestive of pancreatitis[c]) were chosen, a κ of 0.795 (95% confidence interval [CI], 0.69–0.9) was calculated. When a DGGR lipase cutoff >108 U/L and Spec cPL cutoff >400 μg/L (consistent with pancreatitis[c]) were chosen, a κ of 0.551 (CI, 0.43–0.67) was calculated. When a DGGR lipase cutoff >162 U/L (1.5 × the upper reference range) and a Spec cPL cutoff >200 μg/L were chosen, a κ of 0.753 (CI, 0.64–0.86) was calculated. When a DGGR lipase cutoff >162 U/L and a Spec cPL cutoff >400 μg/L were chosen, a κ of 0.774 (CI, 0.67–0.88) was calculated. When applying the same gray zone concept currently used for the Spec cPL to the DGGR lipase assay (109–215 U/L, questionable range; >216, consistent with pancreatitis), a κ value of 0.803 (CI, 0.71–0.9) was calculated for a DGGR lipase cutoff >216 U/L and a Spec cPL cutoff >400 μg/L (Table 2).

The maximal value for κ at a Spec cPL cutoff >200 μg/L was found when the DGGR lipase was set >130 U/L and was calculated as 0.877 (CI, 0.79–0.96). The maximal value for κ at a Spec cPL cutoff >400 μg/L was found when the DGGR lipase was set >190 U/L and was calculated as 0.816 (CI, 0.72–0.91). The Spearman correlation coefficient between both methods of lipase determination was calculated as rho = 0.899.

Agreement between DGGR Lipase and Spec cPL and Pancreatic Ultrasonography

Pancreatic ultrasonography within 24 hours of lipase determinations was performed in 116/142 (81.7%) dogs with suspected pancreatitis. The pancreas could be visualized in 110/116 (94.8%) dogs, and 51/110 (46.4%) dogs had an ultrasonographic diagnosis of pancreatitis. Considering the 51 dogs with an ultrasonographic diagnosis of pancreatitis, Spec cPL was >200 μg/L in 41 (80.4%) and >400 μg/L in 35 (68.6%) dogs, respectively; DGGR lipase activity was >108 U/L in 46 (90.2%) and >216 U/L in 39 (76.5%) dogs, respectively (Table 3).

Table 2. Agreement (κ values, 95% CI) between DGGR lipase and Spec cPL assays.

	Spec cPL >200 μg/L	Spec cPL >400 μg/L
Lipase >108 U/L	0.795 (CI, 0.69–0.9)	0.551 (CI, 0.43–0.67)
Lipase >162 U/L	0.753 (CI, 0.64–0.86)	0.774 (CI, 0.67–0.88)
Lipase >216 U/L	0.699 (CI, 0.58–0.82)	0.803 (CI, 0.71–0.9)

Table 3. Distribution of lipase assay results compared to ultrasonographic diagnosis.

	Ultrasonographic Diagnosis of Pancreatitis (n = 51) (%)	Ultrasonographically Normal Pancreas (n = 59) (%)
Lipase >108 U/L	46/51 (90.2)	35/59 (59.3)
Lipase >216 U/L	39/51 (76.5)	24/59 (40.7)
Spec cPL >200 μg/L	41/51 (80.4)	32/59 (54.2)
Spec cPL >400 μg/L	35/51 (68.6)	25/59 (42.4)

When assessing the agreement between an ultrasonographic diagnosis of pancreatitis and DGGR lipase cutoffs >108 U/L and >216 U/L, respectively, κ values of 0.293 (CI, 0.14–0.44) and 0.352 (CI, 0.18–0.52) were calculated. The maximal value for κ was found when the DGGR lipase cutoff was set >750 U/L and was calculated as 0.446 (CI, 0.28–0.61). When assessing the agreement between an ultrasonographic diagnosis of pancreatitis and a Spec cPL cutoff >200 and >400 μg/L, respectively, κ values of 0.246 (CI, 0.08–0.41) and 0.270 (CI, 0.09–0.45) were calculated. The maximal value for κ was found when the Spec cPL cutoff was set >650 μg/L and was calculated as 0.326 (CI, 0.15–0.5). The agreements of the single pancreatic ultrasonographic variables with results of the DGGR lipase at both cutoffs (>108 U/L, >216 U/L) as well as with results of the Spec cPL at both cutoffs (>200 μg/L, >400 μg/L) are shown in Table 4. For the DGGR lipase, the best agreement (κ, 0.282; CI, 0.14–0.43) was found between an enlarged pancreas and a cutoff >216 U/L. For Spec cPL, the best agreement (κ, 0.232; CI, 0.1–0.37) was found between a hypoechoic pancreas and a cutoff >400 μg/L.

Serum Albumin Concentrations and Ultrasonographic Pancreatic Findings in Hypoalbuminemic Dogs

The mean serum albumin concentration of all 142 dogs was 31 g/L (SD 6.2 g/L), the median albumin concentration was 32 g/L (reference range, 29–37 g/L). A total of 7 dogs (4.9%) had serum albumin concentrations <20 g/L (range, 12–19 g/L; median, 17 g/L): Three out of the 7 dogs were considered to have normal findings on pancreatic ultrasonography, 1 dog did not have ultrasonography performed, and in 1 dog the pancreas could not be identified on ultrasonography. An enlarged pancreas together with a mixed pancreatic echogenicity was found in 2 dogs, 1 of them also had a hyperechoic mesentery. The final ultrasonographic diagnosis was pancreatitis in both of these dogs. A hypoechoic pancreas was found in 21 of 110 (23.1%) dogs on pancreatic ultrasonography. Eighteen of these 21 dogs had Spec cPL concentrations >400 μg/L (median, 705 μg/L) as well as increased DGGR lipase results (median, 793 U/L). Two dogs had Spec cPL >200 μg/L (268 and 225 μg/L, respectively) as well as increased DGGR lipase results (155 and 143 U/L, respectively). One dog had normal lipase results (30 μg/L and 22 U/L). Six of the 21 dogs with pancreatic hypoechogenicity had hypoalbuminemia. The dog with the lowest serum albumin concentration (19 g/L) had an ultrasonographic diagnosis of pancreatitis (eg, hypoechoic pancreas, hyperechoic mesentery, no peritoneal fluid), a Spec cPL >1,000 μg/L, and a DGGR lipase of 2,942 U/L.

Histopathology

Pancreatic histopathologic assessment and corresponding lipase results measured within 4 days of pancreatic tissue sampling were available for 5 dogs

Table 4. Agreements (κ value and 95% CI) of the ultrasonographic diagnosis of pancreatitis and single pancreatic ultrasonographic parameters with results of the DGGR lipase at 2 cutoffs (>108 U/L, >216 U/L), as well as with results of the Spec cPL at 2 cutoffs (>200 μg/L, >400 μg/L).

	Ultrasonographic Diagnosis of Pancreatitis (n = 51)	Hypoechoic Pancreas (n = 21)	Hyperechoic Pancreas (n = 4)	Mixed-Echoic Pancreas (n = 25)	Hyper-echogenic Mesentery (n = 23)	Enlarged Pancreas (n = 31)	Peritoneal Effusion (n = 13)
DGGR lipase >108 U/L	0.293 (0.14–0.44)	0.131 (0.05–0.21)	0.027 (0–0.05)	0.136 (0.04–0.23)	0.111 (0.02–0.2)	0.160 (0.05–0.27)	0.094 (0.04–0.15)
DGGR lipase >216 U/L	0.352 (0.18–0.52)	0.199 (0.08–0.32)	0.023 (0–0.08)	0.292 (0.16–0.42)	0.162 (0.03–0.29)	0.282 (0.14–0.43)	0.149 (0.05–0.25)
Spec cPL >200 μg/L	0.246 (0.08–0.41)	0.180 (0.08–0.28)	–0.019 (0–0.08)	0.163 (0.05–0.27)	0.141 (0.03–0.25)	0.168 (0.04–0.3)	0.096 (0.02–0.17)
Spec cPL >400 μg/L	0.270 (0.09–0.45)	0.232 (0.1–0.37)	–0.006 (0–0.07)	0.227 (0–1.66)	0.229 (0.09–0.37)	0.220 (0.06–0.38)	0.171 (0.06–0.28)

(4 surgical biopsies, 1 necropsy result). Purulent necrotizing pancreatitis was diagnosed in 4 cases, and all dogs had increased DGGR lipase activity ranging from 691 to 4,239 U/L and Spec cPL concentrations ranging from 555 to >1,000 μg/L. The only available necropsy report indicated severe, multifocal, purulent pancreatitis (DGGR lipase 1,046 U/L; Spec cPL, 406 μg/L).

Discussion

The results of this study show high agreement of the catalytic DGGR lipase assay with the Spec cPL, the test commonly regarded as being most sensitive. The present results in dogs indicate even better agreement between the 2 methods than those recently published for cats.[15] On the other hand, the agreement between pancreatic ultrasonography and lipase methods was only fair.

The existing evidence for the mediocre performance of catalytic lipase assays in dogs with pancreatitis is based on studies that have used either the 1,2 diglyceride assay[2,5,6,12] (that also is used by major commercial laboratories[b,f]) or assays that are no longer available[10,23] or studies in which the methodology was not specifically mentioned.[1,24] A recent publication comparing this widely used commercial 1,2DiG assay with the DGGR lipase assay found only poor correlation (rho = 0.25).[25] It is likely that the 1,2-diglyceride assay is not useful for diagnosing pancreatitis in dogs and that usage of this assay most likely has contributed to the generally poor perception of traditional catalytic lipase assays.

Over the years, we noticed striking agreement between pancreatic-specific lipase (cPLI/Spec cPL) and the in-house DGGR lipase results, which led to the initiation of this study. We decided to focus exclusively on comparison of DGGR lipase with Spec cPL, because the Spec cPL test represents the currently available assay. Cohen's kappa coefficient was used to assess agreement between both lipase methodologies because correlations assess the degree of relationship in the data set, but the true aim of method comparison was to assess agreement beyond the data set, between the measurements in any conceivable situation.[26] The interpretation of Cohen's kappa values given by Landis and Koch[22] is somewhat arbitrary, but we believed it would provide some guidance in interpreting kappa values.

When comparing DGGR lipase at a cutoff >108 U/L to Spec cPL at a cutoff >200 μg/L and at a cutoff >400 μg/L, κ decreased from 0.795 (CI, 0.69–0.9) to 0.551 (CI, 0.43–0.67). Although we cannot prove it with this study design, 2 scenarios are plausible. On the one hand, the decreased κ value could be because of an increased number of false-negative Spec cPL test results at a cutoff >400 μg/L. This is likely when considering the wide range of sensitivities reported for the Spec cPL assay for milder forms of pancreatitis.[6,13] On the other hand, the decreased κ value could be because of an increased number of true negatives using the Spec cPL test, if the DGGR lipase results at a cutoff >108 U/L included too many false positives. Without actually knowing for certain the presence or absence and severity of disease, these considerations reflect the limitation of an approach comparing the agreement of 2 methods. Because of this discrepancy in κ values at different Spec cPL concentrations, we chose to integrate a 2-fold DGGR lipase "gray zone" similar to the currently used approach to interpret Spec cPL results[e] into the calculation of agreements between both assays. Interestingly, higher agreements (κ = 0.774 and 0.803, respectively) with spec cPL >400 μg/L were found for the DGGR lipase at cutoffs of 162 and 216 U/L, respectively. Even though data supporting these "gray zone" Spec cPL cutoff results have not been disclosed by the manufacturer, it is conceivable that, analogously to Spec cPL,[27] high intraindividual variabilities (into the abnormal range) also exists for the DGGR lipase in healthy dogs. Future studies must investigate this possibility. Also, because it is virtually impossible to prove that sporadic and transient mild pancreatitis does not exist in clinically healthy dogs, the focus of attention when considering our kappa analyses should be directed at the combined low and high cutoffs of both tests: >108 U/L to >200 μg/L cutoff (κ = 0.795) and >162 or 216 U/L to >400 μg/L cutoff (κ = 0.774 and 0.803). Currently, it is not known which assay yields better diagnostic accuracy. For further evaluation of the 2 lipase assays, studies comparing their results to a defined gold standard or approaches such as Bayesian models capable of dealing with imperfect diagnostic tests are needed. This is especially true when considering the marked cost difference between the 2 methods (ie, currently, the cost of Spec cPL is >10 times the cost of the DGGR lipase at the authors' institution). This cost differential represents a major disadvantage for both the client and the clinician. Another consideration is turnaround time; results of the DGGR lipase are available in 1 hour compared to the Spec cPL which requires 1–2 days.

Ultrasonography may serve as a reliable diagnostic tool for animals with more severe pancreatic pathology. In 1 study, 6 of 9 dogs with pancreatitis that underwent abdominal ultrasonography had ultrasonographic evidence of pancreatitis; these 6 dogs also had the highest histologic pancreatitis activity index.[13] However, ultrasonographic findings were not compared to serum lipase results in that study. We found only slight to fair agreement between pancreatic ultrasonography results and serum lipase results. The overall agreement (ultrasonographic diagnosis of pancreatitis: yes/no) was slightly better for ultrasonography compared to DGGR lipase activity results at the calculated cutoffs, but the κ values obtained still were too low to be clinically useful. Also, no definitive statement concerning higher agreements of ultrasonography with the DGGR lipase is possible, considering the 95% CI. When looking at all evaluated variables, it is noteworthy that κ values always improved when the higher cutoff was used for the 2 lipase results (Table 4). Assuming that higher lipase cutoffs imply higher probabilities of concurrent pancreatitis, this

finding supports the applicability of the evaluated ultrasonographic variables. An interesting exception is the variable 'hyperechogenic pancreas' where the κ values between ultrasonography and lipases actually decrease when the lipase cutoffs are increased. Hyperechoic pancreatic lesions might illustrate a more chronic and potentially healed process with little or no enzyme leakage from acinar cells. Pancreatic edema with anechoic fissures within the pancreas can be associated with hypoalbuminemia or portal hypertension because of fibrotic or vascular liver disease.[28] In that study, the serum albumin concentrations of dogs without evidence of portal hypertension were considerably lower than the lowest concentration found in our study. Although a definitive statement cannot be made (because colloid osmotic pressure was not evaluated in our study), we do not believe that hypoalbuminemia played a major role in the ultrasonographic assessment, given the results of hypoalbuminemic dogs. Ascites caused by portal hypertension was not identified in any of the dogs enrolled in this study.

We focused on ultrasonographic variables that generally are recognized as compatible with pancreatitis.[16,19] Only a few older studies, however, have examined the validity of these variables.[1,17,29,30] Unfortunately, the study using the most accurate approach, in which the pancreas of 73 dogs was sectioned every 2 cm to identify pancreatitis, had no ultrasonographic data available for comparison.[4] The largest previous study on acute necrotizing pancreatitis suspected pancreatitis when the pancreas was hypoechoic and the peripancreatic mesentery was hyperechoic,[1] but the accuracy of ultrasonography in the diagnosis of chronic pancreatitis currently remains unknown.[19]

Although state-of-the-art imaging technology was used in this study, the agreement between commonly accepted laboratory surrogate markers of pancreatitis and ultrasonographic findings still was low. With the study design used, it remains unclear which diagnostic tool performs better but the 2 modalities do not seem interchangeable. There are several possible explanations for the observed discrepancy between ultrasonographic and lipase results. Firstly, it could be that we had too many false-positive ultrasonographic diagnoses of pancreatitis. In this context, an often overlooked factor is the limited information gained from the patients' medical histories. Previous bouts of pancreatitis could either have been clinically silent, misdiagnosed, or simply forgotten during consultations, but remnant pancreatic parenchymal lesions still can be detectable ultrasonographically. A similar argument would be the scenario of silent chronic pancreatitis without clinically relevant enzyme leakage because of fibrous pancreatic remodeling. Other aspects are age- or breed-related pancreatic parenchymal changes. In a recent study on pancreatic ultrasonography in clinically normal dogs, nonhomogenous echotextures or the presence of hyperechoic foci were found predominantly in dogs ≥12 years.[31] However, because different breeds have different age expectancies, this remains difficult to assess. Secondly, it could be that there were too many false-negative ultrasonographic results in patients that truly had pancreatitis. Recognizable ultrasonographic changes might lag behind and depend on when in the course of pancreatitis the patient is presented, which again relates to the usually unknown stage of disease. Aside from that, it has been shown that pancreatitis in dogs can be highly localized,[4] and in the same study gross lesions were very rarely identified during macroscopic examination. This observation together with the recent finding of very low detection rates of single parts of the pancreas in healthy dogs[31] indicates why ultrasonography might miss pancreatitis in dogs. Even though it is known that Spec cPL concentrations do not correlate well with the overall severity of pancreatitis,[6,13] correlations between the extent of enzyme release and size of inflammatory pancreatic foci remain unknown and would require study of experimentally induced pancreatitis, which ethically is not justifiable. Even if focal disease is not the decisive factor, there simply may be insufficient differences between acoustic impedance of abnormal and normal pancreas to permit clinically applicable characterization of pancreatic tissue using current technology. Similar conclusions have been drawn in studies on ultrasonographic findings in liver disease.[32] We do not believe the short half-life of enzymes (as recently demonstrated for Spec cPL[g]) interfered with our results because we only included cases where the time interval between ultrasonography and lipase measurements was <24 hours.

Our study had some limitations. Because of the retrospective study design, ultrasound examinations were carried out by multiple radiologists, who did not follow a standardized ultrasonographic protocol. Although only complete examinations containing all variables were included, it remains unclear how much weight different radiologists placed on single pancreatic findings when a diagnosis of pancreatitis was made. However, under the premise that increased serum lipase results reflect pancreatic pathology, and considering that the highest κ values were calculated for the final radiologic diagnosis of pancreatitis compared to κ values for the single ultrasonographic variables, this might not be a major drawback. In this study, the evaluated ultrasonography report was written by the responsible radiologist immediately after completion of the examination. This approach seems more appropriate than reviewing stored ultrasound images,[5] because it was shown recently that radiologists differ markedly (even between normal and abnormal) in their assessment of static ultrasound images.[32] Ultrasonography reports neither specified how much of both pancreatic limbs and body actually were examined or if body weight affected visibility. This might be a limitation, especially in dogs with an unremarkable ultrasonographic appearance of the scanned pancreas. Notwithstanding these limitations, we believe our approach reflects clinical practice in a referral center. Another limitation is the lack of a gold standard, but there is no agreement and continuing debate as to what constitutes the best gold standard for pancreatitis. Histopathology often is used as a

gold standard, but is not without shortcomings,[4] and it is invasive and impractical for routine use. Ideally, a clinical disease activity scoring system would be used, but such a system would need to be validated first.[33]

In conclusion, the results of this study suggest that there is high agreement between results of the Spec cPL and DGGR lipase tests. The DGGR assay appears to be as useful a method as the Spec cPL and more attractive in terms of cost. Future studies incorporating a defined gold standard are needed to specify the ideal diagnostic cutoffs for the DGGR lipase, because present recommendations to rely only on 2- to 3-fold increases are based on the 1,2 diglyceride lipase assay and cannot be used for DGGR lipase activity. Pancreatic ultrasonography and lipase results only show poor agreement. Patients with clinical signs attributable to pancreatitis still will benefit from a full abdominal ultrasonographic examination to exclude other differential diagnoses, but pancreatic ultrasonography findings need to be interpreted carefully, especially considering the well-established high specificity of the Spec cPL test.[7]

Footnotes

[a] Lipase colorimetric for Roche Cobas Integra 800, Roche Diagnostics, Rotkreuz, Switzerland
[b] IDEXX GmbH Ludwigsburg, Germany
[c] Aloka Prosound SSD alpha 10, U22 xMATRIX ultrasound system, Switzerland Philips AG Healthcare
[d] IBM SPSS v.20 for Mac OS X, IBM Corporation, NY
[e] http://vetmed.tamu.edu/gilab/service/assays/pli
[f] www.antechdiagnostics.com
[g] Dossin O., Rick M., Ridge T.K., Williams D.A., Gruetzner N., Suchodolski J.S., Lefebrve H.P., Steiner J.M. Pharmacokinetics of pancreatic lipase in healthy dogs. Seville, Spain: European College of Veterinary Internal Medicine Congress, September 8–10, 2011:238 (abstract)

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