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Diagnostic utility and validity of 1,2-*o*-dilauryl-*rac*-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase activity in horses with colic

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ABSTRACT

Little is known about the clinical usefulness in horses of the 1,2-*o*-dilauryl-*rac*-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase assay, a biomarker used in other species for the detection of pancreatitis. The main objectives of this study were to evaluate the prevalence of increased DGGR-lipase activity in horses with signs of colic and investigate its association with, and validity to diagnose, underlying gastrointestinal diseases, treatment method (medical or surgical), and outcome (survival or non-survival). Clinical data from 192 horses presented for colic to a teaching hospital were analysed retrospectively. DGGR-lipase activity was measured in frozen plasma collected within 24 h of presentation. Non-parametric tests and Chi-squared or Fisher's exact tests were used to evaluate differences and associations between DGGR-lipase activity and continuous and categorical variables or outcomes, respectively. Measures of the validity of DGGR-lipase as a diagnostic test were also calculated. Increased DGGR-lipase activity above published reference limits was demonstrated in 30.2% of horses with signs of colic, and was above 2x the upper reference limit (URL) in 15.6%. The median DGGR-lipase activity in horses with large bowel displacement or torsion was significantly higher than the median activity for large bowel impaction and for gastric impaction, dilation, or ulceration. DGGR-lipase activity > 2x URL was significantly associated with surgical treatment, strangulating disease, and non-survival. However, as a diagnostic or screening test for these target outcomes, DGGR-lipase activity was poor to fair consequent to poor sensitivity, poor negative likelihood ratio, and an area under the receiver operating characteristic curve, with optimal cut-offs based on the Youden Index, within reference limits.

Introduction

Studies investigating the diagnostic potential of pancreatic enzyme activities, such as lipase and amylase, in horses are scarce and these analytes are not routinely included in equine biochemistry profiles (Grulke et al., 2003; Lorenzo-Figueras et al., 2007). Many veterinary clinical laboratories currently use the chromogenic lipase assay, using the substrate 1,2-*o*-dilauryl-*rac*-glycero-3–glutaric acid-(6'--methylresorufin) ester (DGGR), to measure lipase activity in small animals (Graca et al., 2005). Although specificity is poor, this assay has a sensitivity of 73–93% to diagnose acute pancreatitis in dogs (Graca et al., 2005; Cridge et al., 2018).

However, little is described about the characteristics or diagnostic validity of this assay in horses. A recent study demonstrated that DGGR-lipase was predominantly active in pancreatic tissue in horses, suggesting high tissue specificity (Johnson et al., 2019). In contrast, DGGR-lipase activity increased mildly, although statistically significantly, in dogs and cats after administration of heparin, suggesting that the DGGR-lipase assay also detects lipases of non-pancreatic origin (Lim et al., 2020). Increased DGGR-lipase activity above an upper reference limit (URL) of 20 U/L was determined in 40% of hospitalised horses, the majority of which were diagnosed with gastrointestinal diseases (Johnson et al., 2019). Although the presence of hyperlipasaemia led to the presumption of concurrent pancreatitis secondary to gastrointestinal disease in most of these cases, histopathologic examination was not performed to confirm this diagnosis. Furthermore, pancreatitis, based on gross post-mortem and histologic examination, was diagnosed in only 4/834 (0.4%) horses necropsied over nine years (Newman, 2015) and was diagnosed via necropsy in only 43 cases in a 25-year period at another large teaching hospital (Yamout et al., 2012). In the latter study, pancreatitis occurred most frequently secondary to gastrointestinal disease, particularly large bowel torsion and small intestinal strangulation.

In dogs, hyperlipasaemia may be observed in a variety of non-

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pancreatic diseases, including portal hypertension, gastrointestinal, renal, and endocrine disorders (Rallis et al., 1996; Prümmer et al., 2020; Serrano et al., 2021), and has been associated with prolonged hospitalisation and decreased survival (Prümmer et al., 2020). However, associations between non-pancreatic, particularly gastrointestinal, diseases and hyperlipasaemia and the validity of DGGR-lipase activity to diagnose gastrointestinal disease outcomes have not been investigated in horses.

The aims of the present study were to (1) evaluate the prevalence of elevated DGGR-lipase activity in horses presented for colic, (2) investigate associations between DGGR-lipase activity and outcome (survival or non-survival), treatment type (medical or surgical), and cause of colic, and (3) evaluate the validity of DGGR-lipase as a diagnostic test for survival, the need for surgical treatment, the presence of a strangulating lesion, and the underlying diagnosis category in horses with signs of colic.

Materials and methods

Partial analytical validation of the DGGR-lipase assay

Analytical validation was performed following the American Society for Veterinary Clinical Pathology's guidelines for quality assurance in veterinary clinical pathology (Arnold et al., 2019). Three samples corresponding to low (~12 U/L; within published reference limits or <21 U/L [Desjardins et al., 2017; Johnson et al., 2019]), moderately increased (~80 U/L or ~4x URL), and markedly increased DGGR-lipase activity (~300 U/L or >10x URL) were used. Samples originated from a 10-year-old mare of unknown breed, a 19-year-old warmblood gelding, and a 15-month-old Swiss warmblood colt. Linearity was assessed in samples of ascending dilution prepared by mixing samples with low and high DGGR-lipase activity in different proportions. Repeatability or intra-assay replication was evaluated by measuring all three activity levels in 25 replications within the same run. Reproducibility or inter-assay replication was calculated by measuring each of the levels in five replicates on 5 consecutive days. To avoid multiple freeze-thaw cycles, samples were prepared on day 1 and stored at 4 °C until analysis (up to 4 days).

Animals, samples, data retrieval and management

The database of the Clinical Diagnostic Laboratory of the Vetsuisse Faculty Bern, Switzerland was searched for equine blood samples received during a one-year period (May 2015 to April 2016). The corresponding case files were retrieved from the medical records database of the Swiss Institute of Equine Medicine of the Vetsuisse Faculty Bern, a teaching hospital consulting on predominantly privately-owned leisure horses referred from across Switzerland and bordering regions of neighbouring countries. Horses were included if they were presented for colic, regardless of the underlying cause, if blood was submitted to the diagnostic laboratory for biochemical testing within 24 h of admission, and the remaining plasma sample, frozen at − 25 °C, was available. For horses presented for colic on more than one occasion, only the last visit was included. Institutional animal care and use committee approval for this study was not required as all blood samples were collected for routine diagnostic workup. Consent was obtained from the owners for the use of leftover biological material for research purposes.

DGGR-lipase activity was measured within 12 months of sample submission using the DGGR assay (LIPC, Roche) on a routine biochemistry analyser (Cobas c501, Roche). DGGR-lipase activities were considered increased if they were above an URL of 21 U/L based on available literature (Desjardins et al., 2017; Johnson et al., 2019). However, in this study, horses were classified as having either hyperlipasaemia, defined as > 2x URL (>42 U/L), or normolipasaemia, defined as DGGR-lipase activity ≤ 2x URL (≤42 U/L). The cut-off of 2x URL was chosen to avoid minimally increased activities, which may be clinically insignificant.

All medical records were reviewed by the first author (S.L.). Data retrieved included signalment, length of hospitalisation (LOH), outcome, which was categorised as either survival to discharge or non-survival (i.e., deceased or euthanasia), treatment type (medical or surgical), and clinical diagnosis. Clinical diagnoses were categorised by anatomic location, namely large bowel, small bowel, gastric and other, as well as disease type, namely strangulating or non-strangulating bowel disorders. Clinical diagnoses were also grouped into seven diagnosis categories, as follows: (1) large bowel displacement or torsion; (2) large bowel impaction; (3) large bowel tympany; (4) small bowel impaction or strangulation; (5) enterocolitis; (6) gastric impaction, dilation, or ulceration; and (7) others/unknown. Horses with multiple concurrent disorders were classified by the disorder and location most relevant as the cause of colic. To help establish a definitive diagnosis and rule in or out pancreatic pathology, post-mortem examination findings were retrieved from necropsy reports, where available. Deceased horses were submitted for post-mortem examination at the clinician's discretion and with owner consent, predominantly in cases where the underlying cause for colic could not be fully established.

Statistical methods

Data were analysed using commercial software .[1] For assay validation, linearity was evaluated visually by plotting expected values and mean measured values, and with regression analysis. Mean, standard deviation, and intra- and inter-assay coefficients of variation were calculated for repeatability and reproducibility.

Normality of data from clinical cases was assessed using D'Agostino-Pearson tests and by reviewing Q-Q plots. As continuous data were non-normally distributed, results were reported as median (minimum, interquartile range [IQR], maximum). Chi-squared or Fisher's exact test, where appropriate, and odds ratios (OR) with 95% confidence intervals (CI) were used to evaluate associations between horses with hyperlipasaemia or normolipasaemia and categorical data. Mann-Whitney *U* tests were used to evaluate differences in continuous data (i.e., age and LOH) between horses with hyperlipasaemia or normolipasaemia. Kruskall-Wallis tests, followed by Dunn's post hoc test for multiple comparisons were used to evaluate differences between DGGR-lipase activity as a continuous variable and categorical data. Sensitivity, specificity, positive and negative predictive values, pre-test and post-test probabilities, and positive and negative likelihood ratios were calculated for DGGR-lipase activity as a diagnostic test. Hyperlipasaemia (DGGR-lipase activity >2x URL or >42 U/L) was considered the positive test and non-survivors, surgical treatment, strangulating disease, and large bowel displacement or torsion were considered the positive target outcomes. The area under the receiver operating characteristic (AUROC) curve was also plotted across these target outcomes and by using the Youden index, the optimal cut-offs and sensitivities and specificities for DGGR-lipase as a diagnostic test were determined. In addition, sensitivities and DGGR-lipase activity cut-offs, given a set specificity of 80%, 90% and 95%, were calculated along with bootstrapped 95% CI. Statistical significance was set at $P \leq 0.05$.

Results

Partial analytical validation of the DGGR-lipase assay

The assay was linear in the range 12–306 U/L ($r^2 = 0.9998$) with < 2.3% deviation from the calculated value at any point. Within-run coefficients of variation were 4.43%, 0.69%, and 1.00% for the low, medium, and high samples, respectively. Between-run coefficients of

[1] See: MedCalc, version 19.2.1. https://www.medcalc.org (Accessed 2 September, 2022)

variation for the low, medium, and high samples were 3.57%, 1.42%, and 1.16%, respectively.

Descriptive statistics

In total, 192 horses (89 mares, 95 geldings, eight stallions) met the inclusion criteria. There were 43 different breeds including 57 Swiss Warmbloods (29.7%), 20 Freibergers (10.4%), 14 Selle Français (7.3%), nine Shetland ponies (4.7%), six Hanoverians and Dutch Warmbloods (3.1% each), five Holsteiners and Lusitanos (2.6% each), and less than five horses of other breeds. The age at presentation was available for 190 cases, with a median of 12.0 years (IQR 7.0–16.2 years). The median LOH was 4 days (IQR 3–8 days; Table 1).

A total of 48/192 (25.0%) horses underwent surgery and 161 (83.9%) horses survived to discharge (Table 1), including 124/144 (86.1%) medical and 37/48 (77.1%) surgical cases. Large bowel disorders were most prevalent (128/192; 66.7%), with large bowel impaction being the most common clinical diagnosis category (67/192; 34.9%), whereas 39/192 (20.3%) were diagnosed with large bowel displacement or torsion (Tables 1 and 2). The group 'other' (n = 14 in Table 2) included four cases of peritonitis, one of grass sickness, one hepatopathy, one uterine torsion, and seven cases where a final diagnosis could not be reached. Of the horses with a diagnosis, 29/185 (15.7%) had strangulating bowel disorders, including 22 small bowel strangulations and seven colon torsions.

Compared with horses with normolipasaemia, horses with hyperlipasaemia had a higher percentage of non-survivors, surgical treatment, strangulating disease, and large bowel displacement or torsion (Table 1).

Post-mortem examination was performed in 10 cases, comprising five horses with hyperlipasaemia, including the horse with the highest value. No macroscopic abnormalities of the pancreas were identified in any of the cases, and consequently no histopathological examinations of the pancreas were performed.

Prevalence of increased DGGR-lipase activity

DGGR-lipase activity for all 192 horses ranged from 3 to 2126 U/L with a median of 15 U/L (IQR 11–26 U/L). Values were within published reference limits in 134 (69.8%), between 1x and 2x URL in 28 (14.6%), and above 2x URL in 30 (15.6%) horses. The overall prevalence of increased DGGR-lipase activity was therefore 30.2% (58 of 192 horses) in which 30 horses (15.6%) were assigned to the hyperlipasaemia group as defined in this study.

Table 2
Results of the median DGGR-lipase activities (U/L) and the number and percentage (%) of horses across the clinical diagnosis variables and categories in 192 horses with signs of colic.

Clinical diagnosis variables and categories		Horses, n (%)	DGGR-lipase activity, U/L median (min, IQR, max)	P
Anatomic location	Large bowel	128 (66.7%)	15 (4, 11–27, 2126)	0.226
	Small bowel	28 (14.6%)	16.5 (3, 12–33, 1211)	
	Gastric	22 (11.5%)	12 (9, 11–18, 156)	
	Other	14 (7.3%)	12.5 (6, 9–24, 125)	
Disease type	Strangulating[a]	29 (15.7%)	24 (11, 14.8–107.5, 1211)	< 0.001
	Non-strangulating[a]	156 (84.3%)	14 (3, 11–3, 2126)	
Diagnosis category	Large bowel displacement or torsion[b,c]	39 (20.3%)	27 (9, 14.0–82.5, 1801)	< 0.001
	Large bowel impaction[b]	67 (34.9%)	12 (8, 10–19, 2126)	
	Large bowel tympany	13 (6.8%)	16 (4, 13.8–24.5, 223)	
	Small bowel impaction or strangulation	28 (14.6%)	16.5 (3, 12–33, 1211)	
	Enterocolitis	9 (4.7%)	11 (10, 10.8–19.5, 27)	
	Gastric impaction, dilation, or ulceration[c]	22 (11.5%)	12 (9, 11–18, 156)	
	Other	14 (7.3%)	12.5 (6, 9–24, 125)	

IQR, interquartile range; min, minimum; Max, maximum; U/L, units per litre
[a, b, c] Categories within variables that differed significantly from each other (P < 0.05) are denoted by the same superscript letters.

Statistical analyses

There was no significant difference in the median age (years) or LOH (days) between horses with normolipasaemia and hyperlipasaemia, and there was no association between sex and DGGR-lipase activity (Table 1). Non-survivors were significantly more likely to have hyperlipasaemia than survivors (P = 0.029). Horses undergoing surgery were almost eight times more likely to have hyperlipasaemia than horses

Table 1
Results of descriptive statistics, median differences, and associations (odds ratios [OR] and 95% confidence intervals [CI]) between DGGR-lipase activity categories across relevant variables in 192 horses presented for signs of colic. Hyperlipasaemia was defined as DGGR-lipase activity > 2x URL (>42 U/L) and normolipasaesmia as DGGR-activity ≤ 2x URL (≤42 U/L).

Variable		Total (n = 192)	DGGR-lipase activity, U/L			
			Hyperlipasaemia (n = 30)	Normolipasaemia (n = 162)	OR (95% CI)	P
Age, median years (min, IQR, max)		12.0 (0.1, 7.0–16.2, 38.7)	12.1 (0.3, 9.8–18.0, 3.2)	11.8 (0.1, 6.8–15.9, 38.7)		0.275
LOH, median days (min, IQR, max)		4 (1, 3–8, 59)	6 (1, 3–9, 34)	4 (1, 3–7, 59)		0.198
Sex n (%)	Male	103 (53.6%)	18 (60%)	85 (52.5%)		0.449
	Female	89 (46.4%)	12 (40%)	77 (47.5%)		
Outcome n (%)	Non-survival	31 (16.1%)	9 (30.0%)	22 (13.6%)	2.7 (1.1–6.7)	0.029
	Survival	161 (83.9%)	21 (70%)	140 (86.4%)		
Treatment n (%)	Surgical	48 (25.0%)	19 (63.3%)	29 (17.9%)	7.9 (3.4–18.4)	< 0.001
	Medical	144 (75.0%)	11 (36.7%)	133 (82.1%)		
Disease type n (%)[a]	Strangulating	29 (15.7%)	9 (31.0%)	20 (12.8%)	3.1 (1.2–7.7)	0.017
	Non-strangulating	156 (84.3%)	20 (69.0%)	136 (87.2%)		
Disease category n (%)	Large bowel displacement or torsion	39 (20.3%)	15 (50.0%)	24 (14.8%)	5.8 (2.5–13.3)	< 0.001
	Other categories	153 (79.7%)	15 (50.0%)	138 (85.2%)		

CI, confidence interval; IQR, interquartile range; LOH, length of hospitalisation; min, minimum; Max, maximum; OR, odds ratio; U/L, units/L; URL, upper reference limit.
[a] Information was available for 185 horses (29 with hyperlipasaemia and 156 that were normolipasaemic).

requiring medical treatment (OR, 7.9; 95% CI, 3.4–18.4). Horses with a strangulating lesion were significantly more likely to have hyperlipasaemia than those with non-strangulating lesions (OR, 3.1; 95% CI, 1.2–7.7), whereas horses with large bowel displacement or torsion were almost six times more likely to have hyperlipasaemia than those with other disease categories (OR, 5.8; 95% CI, 2.5–13.3; Table 1).

The median DGGR-lipase activities across the three clinical diagnosis variables, anatomic location, disease type, and diagnosis categories are summarised in Table 2. There was no difference in the median DGGR-lipase activity between horses grouped by anatomic disease location. However, there was a significant difference in median DGGR-lipase activities between horses with strangulating versus non-strangulating disease ($P < 0.001$), as well as between horses in different diagnosis categories ($P < 0.001$). Post hoc analysis demonstrated that the median DGGR-lipase activity in horses with large bowel displacement or torsion (median, 27 U/L; IQR 14.0–82.5 U/L) was significantly higher than both large bowel impaction (median, 12 U/L; IQR 10–19 U/L) and gastric impaction, dilation, or ulceration (median, 12 U/L; IQR 11–18 U/L; Table 2).

Table 3 shows the results of the validity of DGGR-lipase activity, using cut-offs for hyperlipasaemia and normolipasaemia as defined, to diagnose survival outcome, treatment, disease type and diagnosis category. Hyperlipasaemia lead to a change from pre-test to post-test probability from 16% to 30% for non-survival, 25 to 63% for surgical treatment, 16–31% for strangulating disease, and 20–50% for large bowel displacement or torsion. The AUROC curve demonstrated that DGGR-lipase activity was a poor to fair diagnostic test for the target variables investigated, with optimal cut-offs based on the Youden Index, within reference limits (Table 4). Higher specificities could be achieved at higher DGGR-lipase activity cut-offs above published reference limits, although only at the expense of sensitivity, which became considerably lower.

Discussion

The results of the validation studies demonstrated that the DGGR-lipase assay used in this study had adequate linearity, repeatability, and reproducibility for use on equine plasma samples, providing a robust basis for its application in equine clinical diagnostics and research.

The results of this study showed that 30.2% of horses presented for signs of colic to a veterinary teaching hospital had increased DGGR-lipase activity above published reference limits. This prevalence was substantially lower than the approximately 61–79% of horses with signs of colic that had hyperlipasaemia reported in a previous study (Johnson et al., 2019). The major reason for this discrepancy was a difference in population characteristics. Specifically, the population in the Johnson et al. (2019) study comprised a larger number of foals (up to a year) and thoroughbreds in training, both unrepresented in the current study, and both of which had a higher prevalence of hyperlipasaemia than adults and other breeds. However, this latter study also reported a lower prevalence of hyperlipasaemia in warm-blooded (e.g., Dutch warmbloods and Standardbreds; 32%) and cold-blooded breeds (e.g., ponies, Friesian, Hunters, and Irish Draughts; 25%), similar to the current study population. Other reasons for the discrepancy in prevalence between the current study and that of Johnson et al. (2019) include differences in the timing of sample collection, the duration and conditions of storage of samples prior to analysis, and the type of DGGR-lipase assay and biochemical analyser used, each of which may have impacted DGGR-lipase activity. Prolonged sample storage (up to 12 months) in the current study, for example, may have decreased the DGGR-lipase activity in vitro, potentially leading to underestimation of hyperlipasaemia. Given these differences in study design, the reference limits established by Johnson et al. (2019) may not have been entirely applicable to the population used in this study and justified the use of > 2x URL as the cut-off to define hyperlipasaemia.

It is important to note that the prevalence of increased DGGR-lipase activity in horses with signs of colic in both this and the Johnson et al. (2019) study was markedly higher than the prevalence of primary pancreatitis reported in necropsy studies (Yamout et al., 2012; Newman, 2015). Given the high tissue specificity of the DGGR-lipase assay for the equine pancreas (Johnson et al., 2019), this finding suggests that hyperlipasaemia in the current study most likely resulted from secondary pancreatic damage rather than from primary pancreatitis (Yamout et al., 2012; Johnson et al., 2019). Studies have reported that large colon torsion and right dorsal displacement were frequently linked with increased DGGR-lipase activity, which was hypothesised to be the result of reduced perfusion to the pancreas and subsequent leakage of pancreatic lipase from damaged exocrine pancreatic acinar cells (Yamout et al., 2012; Johnson et al., 2019). Large bowel displacement or torsion was also the diagnosis category with the highest median DGGR-lipase activity in the current study. In addition, histopathologic and ultrastructural evidence of pancreatic damage were reported in horses with small and large bowel obstruction and was theorised to be caused by ischaemia secondary to shock-related hypoperfusion and mechanical compression (Grulke et al., 2003). Furthermore, non-specific increases in DGGR-lipase activity may also have led to hyperlipasaemia in the absence of clinically overt pancreatitis, as described in dogs with gastrointestinal diseases (Rallis et al., 1996).

A final point of evidence lending support to the likelihood that pancreatic damage occurred secondary to the underlying cause of colic in the horses of this study, rather than primary pancreatitis, was that no gross pancreatic pathology was observed in any of the small number of horses that underwent post-mortem, although no histopathological examination was performed. While little can be concluded about the true nature of the pancreatic damage in this study's population, evaluation of the validity of DGGR-lipase activity as a diagnostic test for primary or concurrent pancreatitis or pancreatic pathology was not the aim of this study. Further research comparing DGGR-lipase activity to a well-defined reference standard, such as histopathologic confirmation of pancreatic pathology, may clarify this issue in future.

Despite median activity of DGGR-lipase being significantly higher in horses with large bowel displacement or torsion than in cases with large bowel impaction or gastric impaction, dilation or ulceration, there was overlap in DGGR-lipase activity between the various diagnosis categories and, except for large bowel displacement or torsion, median DGGR-lipase activities were all below the published reference limit (i.e., <21 U/L). Grouping cases with large bowel displacement and large bowel torsion together may have resulted in higher DGGR-lipase activity given that colon torsion is a strangulating disease, which may have a greater impact on pancreatic perfusion with resultant enzyme leakage (Yamout et al., 2012). However, the low numbers of cases in these subgroups required merging of these categories and precluded their statistical analysis separately.

Similar to studies in dogs (Prümmer et al., 2020), hyperlipasaemia was significantly associated with non-survival. Horses with strangulating diseases and horses undergoing surgical treatment also were significantly more likely to have hyperlipasaemia. Given the evidence for significant negative associations between strangulating gastrointestinal disease, the need for surgery, and survival (Sutton et al., 2009), it is possible that hyperlipasaemia may act as a confounder or be an intervening variable or interact with either the disease process or the disease treatment or both prior to the outcome (i.e., survival or non-survival). However, determination of the potential interrelationships between these variables and specific outcomes in horses with signs of colic was beyond the scope of this cross-sectional study and an appropriately designed epidemiological study conducted to address these aims in future would be beneficial.

The results of this study also demonstrated that DGGR-lipase activity was only a poor to fair diagnostic test to identify large bowel displacement or torsion, non-survivors, the need for surgical treatment, and strangulating gastrointestinal disease, at both the defined

Table 3
Validity of DGGR-lipase activity as a diagnostic test for selected target outcomes in 192 horses presented for signs of colic where DGGR-lipase activity > 2x URL (>42 U/L), or hyperlipasaemia, was defined as the positive test.

Target variables		Horses (*n*)		Total	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	PLR (95% CI)	NLR (95% CI)
		Hyper-lipasaemia	Normo-lipasaemia							
Outcome	Non-survival	9	22	31	29 (14–48)	87 (81–92)	30 (18–46)	86 (83–89)	2.2 (1.1–4.4)	0.8 (0.6–1.0)
	Survival	21	140	161						
Treatment	Surgical	19	29	48	40 (26–55)	92 (87–96)	63 (47–77)	82 (78–85)	5.2 (2.7–10.1)	0.7 (0.5–0.8)
	Medical	11	133	144						
Disease type[a]	Strangulating	9	20	29	31 (15–51)	87 (81–92)	31 (19–47)	87 (84–90)	2.4 (1.2–4.8)	0.8 (0.6–1.0)
	Non-strangulating	20	136	156						
Diagnosis category	Large bowel displacement or torsion	15	24	39	38 (23–55)	90 (84–94)	50 (35–65)	85 (82–88)	3.9 (2.1–7.3)	0.7 (0.5–0.9)
	All other categories	15	138	153						

CI, confidence interval; NLR, negative likelihood ratio, NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; U/L, units per litre; URL, upper reference limit.
[a] Information was available for 185 horses (29 with hyperlipasaemia and 156 that were normolipasaemic).

Table 4
Area under the receiver operating characteristic (AUROC) curve analysis evaluating the diagnostic validity of DGGR-lipase activity for selected target outcomes in 192 horses with signs of colic, including the sensitivity and specificity at optimal cut-offs (based on the Youden-index) and the resultant cut-offs for increasing set specificities.

Target variable	AUROC (95% CI)	Optimal values			80% specificity		90% specificity		95% specificity	
		DGGR-lipase activity cut-off (U/L)	Sensitivity % (95% CI)	Specificity % (95% CI)	DGGR-lipase activity cut- off (U/L)	Sensitivity % (95% CI)	DGGR-lipase activity cut- off (U/L)	Sensitivity % (95% CI)	DGGR-lipase activity cut-off (U/L)	Sensitivity % (95% CI)
Nonsurvival	0.65 (0.57–0.71)	> 14	68 (49–83)	53 (45–61)	> 29	35 (19–55)	> 64	23 (10–45)	> 155	13 (3–38)
Surgical treatment	0.79 (0.72–0.84)	> 18	75 (60–86)	74 (66–81)	> 20	66 (48–81)	> 32	48 (31–68)	> 53	38 (21–58)
Strangulating disease	0.72 (0.65–0.78)	> 12	90 (73–98)	44 (36–52)	> 27	45 (24–66)	> 56	31 (14–52)	> 134	21 (7–41)
Large bowel torsion or displacement	0.73 (0.66–0.79)	> 19	64 (47–79)	73 (65–79)	> 24	52 (36–70)	> 41	39 (13–62)	> 140	15 (3–49)

AUROC, Area under the receiver operating characteristic curve; CI, confidence interval; U/L, units per litre

hyperlipasaemia cut-off (i.e., >2x URL or >42 U/L) and at optimal cut-offs. This was based on low sensitivity, and the low proportion of truly positive tests, and poor negative likelihood ratio, which indicated the inability of a negative test (i.e., normolipasaemia) to reliably rule out horses without the target outcomes. Although higher specificities could be achieved at higher cut-off DGGR-lipase activities, AUROC curve analyses indicated that this occurred at the expense of sensitivity, a highly undesirable outcome when identifying horses with critical gastrointestinal outcomes was the main purpose of this test. In addition, despite moderate to high specificity and negative predictive values indicating a stronger ability of the DGGR-lipase test to correctly identify horses without strangulating disease or the need for surgery, the small percentage of false negative tests would likely still be too high for veterinarians to accept this risk of missing a significant outcome. Therefore, the use of DGGR-lipase activity as a single test alone cannot reliably diagnose or screen equine colic cases in first opinion or referral settings. Given the added burden of higher cost and the need for rapid access to a diagnostic laboratory that uses a standard wet chemistry analyser, the value of including DGGR-lipase activity as a routine test into equine biochemical profiles is debatable at this time. Research examining serial measurements of DGGR-lipase activities over time, or simultaneously alongside other diagnostic tests, and in horses with a wider range of clinical diseases may also help to further evaluate the diagnostic validity of this assay.

The population of horses in this study consisted predominantly of privately-owned horses referred from throughout Switzerland that were used mainly for leisure. Therefore, results of the current study may not be applicable to other equine populations, such as sport horses or racehorses, primary care practices, or to horses from other geographic areas. Other limitations of this study included bias consequent to retrospective data collection where medical records were uncertain of the final diagnosis, particularly in medically treated horses. Lack of standardised timing of blood sampling, which may have occurred after initiating volume replacement therapy and pain relief, may also have resulted in variability and potentially underestimation of DGGR-lipase activity. Despite the large case number being a major strength of this work, no healthy horses were included, which precluded the calculation of population-specific reference limits and may have negatively impacted the validity and utility of DGGR-lipase activity as a diagnostic test in the population of horses used.

Conclusions

This study demonstrated increased DGGR-lipase activity above published reference limits in approximately one third of horses presented with colic, most commonly due to large bowel displacement and torsion. The results demonstrated significant associations between hyperlipasaemia (i.e., >2x URL) and strangulating disease, surgery, and non-survival. However, the validity of DGGR-lipase activity, measured within 24 h of presentation, to diagnose strangulating lesions, the need for surgery, or non-survival was poor to fair, and limits the value of this assay as a screening or diagnostic test in horses with colic.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

Arnold, J.E., Camus, M.S., Freeman, K.P., Giori, L., Hooijberg, E.H., Jeffery, U., Korchia, J., Meindel, M.J., Moore, A.R., Sisson, S.C., et al., 2019. ASVCP Guidelines: principles of quality assurance and standards for veterinary clinical pathology (version 3.0): developed by the American Society for Veterinary Clinical Pathology's (ASVCP) Quality Assurance and Laboratory Standards (QALS) Committee. Veterinary Clinical Pathology 48, 542–618.

Cridge, H., MacLeod, A.G., Pachtinger, G.E., Mackin, A.J., Sullivant, A.M., Thomason, J. M., Archer, T.M., Lunsford, K.V., Rosenthal, K., Wills, R.W., 2018. Evaluation of SNAP cPL, Spec cPL, VetScan cPL Rapid Test, and Precision PSL Assays for the diagnosis of clinical pancreatitis in dogs. Journal of Veterinary Internal Medicine 32, 658–664.

Desjardins, I., Randleff-Rassmussen, P., Rannou, B., 2017. Plasmatic DGGR-lipase activity in 54 horses affected with endocrine diseases. In: Proceedings of the 10th Annual European College of Equine Internal Medicine Congress, Budapest, Hungary, 2nd-4th November 2017.

Graca, R., Messick, J., McCullough, S., Barger, A., Hoffmann, W., 2005. Validation and diagnostic efficacy of a lipase assay using the substrate 1,2-o-dilauryl-rac-glycero glutaric acid-(6' methyl resorufin)-ester for the diagnosis of acute pancreatitis in dogs. Veterinary Clinical Pathology 34, 39–43.

Grulke, S., Deby-Dupont, G., Cassart, D., Gangl, M., Caudron, I., Lamy, M., Serteyn, D., 2003. Pancreatic injury in equine acute abdomen evaluated by plasma trypsin activity and histopathology of pancreatic tissue. Veterinary Pathology 40, 8–13.

Johnson, J.P., Stack, J.D., McGivney, C.L., Garrett, M.P., O'Brien, P.J., 2019. DGGR-lipase for effective diagnosis of pancreatitis in horses. Comparative Clinical Pathology 28, 1625–1636.

Lim, S.Y., Xenoulis, P.G., Stavroulaki, E.M., Lidbury, J.A., Suchodolski, J.S., Carrière, F., Steiner, J.M., 2020. The 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase assay in cats and dogs is not specific for pancreatic lipase. Veterinary Clinical Pathology 49, 607–613.

Lorenzo-Figueras, M., Morisset, S.M., Morisset, J., Laine, J., Merritt, A.M., 2007. Digestive enzyme concentrations and activities in healthy pancreatic tissue of horses. American Journal of Veterinary Research 68, 1070–1072.

Newman, S.J., 2015. Equine pancreatic disease: a review and characterization of the lesions of four cases (2005-2014). Journal of Veterinary Diagnostic Investigation 27, 92–96.

Prümmer, J.K., Howard, J., Grandt, L.M., Obrador de Aguilar, R., Meneses, F., Peters, L. M., 2020. Hyperlipasemia in critically ill dogs with and without acute pancreatitis: prevalence, underlying diseases, predictors, and outcome. Journal of Veterinary Internal Medicine 34, 2319–2329.

Rallis, T.S., Koutinas, A.F., Kritsepi, M., Moraitou, K.T., 1996. Serum lipase activity in young dogs with acute enteritis or gastroenteritis. Veterinary Clinical Pathology 25, 65–68.

Serrano, G., Paepe, D., Williams, T., Watson, P., 2021. Increased canine pancreatic lipase immunoreactivity (cPLI) and 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase in dogs with evidence of portal hypertension and normal pancreatic histology: a pilot study. Journal of Veterinary Diagnostic Investigation 33, 548–553.

Sutton, G.A., Ertzman-Ginsburg, R., Steinman, A., Milgram, J., 2009. Initial investigation of mortality rates and prognostic indicators in horses with colic in Israel: a retrospective study. Equine Veterinary Journal 41, 482–486.

Yamout, S.Z., Nieto, J.E., Anderson, J., De Cock, H.E., Vapniarsky, N., Aleman, M., 2012. Pathological evidence of pancreatitis in 43 horses (1986-2011). Equine Veterinary Journal Supplements 45–50.

[2] See: Figshare. Partial analytical validation data of the DGGR lipase assay in equine plasma https://doi.org/10.6084/m9.figshare.19549339.v1 (Accessed 2 September 2022)