Assessment of serum pancreatic (DGGR) lipase concentrations in equids with gastrointestinal disease

Tim Mair[1], Harry Stone[1], and Imogen Schofield[1]

[1]CVS Group plc

February 07, 2025

Abstract

Background : Pancreatitis is a poorly understood condition in the horse. The DGGR lipase assay has recently been validated for horses. Objectives : Evaluate serum DGGR lipase concentrations in equids presented to an equine hospital in the UK with gastrointestinal disease. Study design : Prospective descriptive. Methods: Blood samples were obtained by convenience sampling of horses and donkeys presented for evaluation of gastrointestinal disease. Results : Serum pancreatic (DGGR) lipase concentrations were measured in 205 equids with gastrointestinal disease, of which 147 survived, 47 were euthanised and 11 died. The median serum pancreatic lipase concentration in all animals was 17 U/l (IQR 14 – 27; range 1 – 3484). The lipase concentration was categorised as normal in 124 animals (60.5%) and elevated in 81 (39.5%). There was a statistically significant difference in the disease category and pancreatic lipase concentration ($p < 0.001$), with colic cases having higher lipase concentrations than colitis and peritonitis cases. There was strong evidence (p=0.01) of an association between pain severity and lipase values, with higher lipase concentrations in horses with more severe pain. Of 12 horses with severely increased pancreatic lipase concentration (>200 u/L) 3/12 had spontaneous nasogastric reflux and 6/10 had distended and/or thickened small intestine on abdominal ultrasonography; 7/12 survived to hospital discharge, and 5/12 died or were euthanised. Main Limitations : We were unable to confirm the presence of pancreatitis in any of the horses with elevated serum DGGR lipase concentrations by post-mortem examination or histopathology. Conclusions : Some equids with gastrointestinal disease have increased serum pancreatic (DGGR) lipase concentrations, especially those with colic. This suggests that a degree of pancreatitis may be present in many colic cases, although this does not necessarily indicate causation.

Assessment of serum pancreatic (DGGR) lipase concentrations in equids with gastrointestinal disease

[1,3]Mair, T.S., [2,3]Stone, H.A. and[3]Schofield, I.

[1]Bell Equine Veterinary Clinic, Mereworth, Kent, ME18 5GS

[2]B&W Equine Vets, Ashton Hill Farm, Failand, Bristol, BS38 3US[3]CVS Group Ltd, CVS House, Owen Road, Diss, Norfolk, IP22 4ER

Abstract

Background: Pancreatitis is a poorly understood condition in the horse. The DGGR lipase assay has recently been validated for horses.

Objectives: Evaluate serum DGGR lipase concentrations in equids presented to an equine hospital in the UK with gastrointestinal disease.

Study design: Prospective descriptive.

Methods: Blood samples were obtained by convenience sampling of horses and donkeys presented for evaluation of gastrointestinal disease.

Results: Serum pancreatic (DGGR) lipase concentrations were measured in 205 equids with gastrointestinal disease, of which 147 survived, 47 were euthanised and 11 died. The median serum pancreatic lipase concentration in all animals was 17 U/l (IQR 14 – 27; range 1 – 3484). The lipase concentration was categorised as normal in 124 animals (60.5%) and elevated in 81 (39.5%). There was a statistically significant difference in the disease category and pancreatic lipase concentration ($p < 0.001$), with colic cases having higher lipase concentrations than colitis and peritonitis cases. There was strong evidence (p=0.01) of an association between pain severity and lipase values, with higher lipase concentrations in horses with more severe pain. Of 12 horses with severely increased pancreatic lipase concentration (>200 u/L) 3/12 had spontaneous nasogastric reflux and 6/10 had distended and/or thickened small intestine on abdominal ultrasonography; 7/12 survived to hospital discharge, and 5/12 died or were euthanised.

Main Limitations: We were unable to confirm the presence of pancreatitis in any of the horses with elevated serum DGGR lipase concentrations by post-mortem examination or histopathology.

Conclusions: Some equids with gastrointestinal disease have increased serum pancreatic (DGGR) lipase concentrations, especially those with colic. This suggests that a degree of pancreatitis may be present in many colic cases, although this does not necessarily indicate causation.

Keywords: Pancreas, pancreatitis, lipase, colic, horse

Clinical Relevance

- Some equids with colic have evidence of pancreatitis as evidenced by elevated serum DGGR (pancreatic) lipase concentrations
- Pancreatitis may be primary or secondary
- Equids with evidence of pancreatitis (elevated serum pancreatic lipase concentrations) tend to show more pain and have a lower survival rate than equids without evidence of pancreatitis

Introduction

Pancreatitis is commonly encountered in people, dogs and cats, but the clinical presentation can vary from subclinical or mild nonspecific clinical signs to severe life-threatening disease (Mansfield 2012a). Acute and chronic forms are recognised, although these may occur concurrently. Understanding of the pathophysiology of acute pancreatitis is largely extrapolated from human clinical studies and experimental animal models (Bjørnkjær-Nielsen and Bjørnvad 2021). It is believed to result from the activation of trypsinogen and release of cathepsin-B and other pancreatic enzymes within acinar cells, leading to the activation of the apoptotic cascade inflammation, which is manifested by neutrophil migration to the pancreas as well as probable complement activation and a "cytokine storm" that further contributes to inflammation (Mansfield 2012b; Talukdar *et al* 2016).

Ante-mortem diagnosis of acute pancreatitis is difficult owing to the presence of nonspecific clinical signs and the lack of definitive diagnostic tests, including enzyme biomarkers (Johnson *et al* 2019). The biomarker ineffectiveness relates to low amylase activity of the equine pancreas (Harris and Gow 1892; Lorenzo-Figueras *et al* 2007), lack of tissue specificity of conventional lipase assays (Tietz and Shuey 1993) and instability of trypsin (Grulke *et al* 2003). In the absence of any one specific clinical sign or combination of signs that have been identified as pathognomonic for acute pancreatitis in dogs and cats (Xenoulis 2015), diagnosis is often based on a combination of clinical signs (such as anorexia, vomiting, weakness, and abdominal pain) and clinical pathology findings (Cridge *et al* 2021).

In most previously reported cases, the confirmation of pancreatitis in horses and donkeys has been established by post-mortem examination (Yamout *et al* 2012). The 1,2-o-dilauryl-rac-glycero-3-glutarate-(6'-methylresorufin) ester (DGGR) lipase assay has been shown to be specific for pancreatic lipase and has recently been validated for horses (Johnson *et al* 2019; Peters and Howard 2023). In the study by Johnson *et al* (2019) from Ireland, venous blood was collected and analysed for pancreatic (DGGR) lipase from 109 hospitalised horses (including 33 horses with various types of gastrointestinal disease, 22 of which had colic); 85% (28/33) of these horses had increased pancreatic lipase concentration. In another study from

Switzerland (Lanz *et al* 2022), increased DGGR-lipase concentration above published reference limits was demonstrated in 30.2% of 192 horses with signs of colic, and was above 2x the upper reference limit (URL) in 15.6%. The median DGGR-lipase concentration in horses with large bowel displacement or torsion was significantly higher than the median concentration for horses with large bowel impaction, gastric impaction, dilation, or ulceration. DGGR-lipase concentration > 2x URL was significantly associated with surgical treatment, strangulating disease, and non-survival.

The aims of this study were to evaluate serum DGGR lipase concentrations in horses and donkeys presented to an equine hospital in the UK with gastrointestinal disease. The objectives were similar to a recently published study from Switzerland (Lanz *et al* 2022): to evaluate the prevalence of elevated DGGR lipase concentrations in horses presented for gastrointestinal disease, and to investigate any associations between DGGR lipase concentration at admission of cases to our hospital and the cause of colic, the outcome (survival or non-survival), and the treatment type (medical or surgical).

Materials and Methods

Serum samples for pancreatic lipase (DGGR substrate) analysis were obtained prospectively by convenience sampling of horses and donkeys presenting to Bell Equine Veterinary Clinic for investigation of gastrointestinal disease, where surplus blood taken for routine clinical evaluation of the cases was available. For the purposes of this study, gastrointestinal disease was defined as equids presenting with clinical signs of acute colic and/or acute diarrhoea. Serum was harvested from clotted blood samples collected from eligible equids on admission to the hospital; in some cases, serum was also collected on consecutive days when blood was being drawn for ongoing clinical management. Consent was obtained from the owners for the use of left-over biological material for research purposes, both by the owners signing the hospital consent form and verbally. Serum was acquired by centrifugation of clotted blood samples and submitted to the laboratory within 24 hours of collection. Ethical approval was provided by the CVS Ethics Committee (CVS-2022-005).

Where available, results of peripheral haematological and plasma biochemical analyses (including white blood cell and neutrophil counts, packed cell volume, total protein and albumin concentrations, and lactate concentrations) and peritoneal total nucleated cell counts and lactate concentrations for samples obtained on admission to the hospital were recorded. The duration of illness prior to admission and severity of abdominal pain on admission were recorded, as were the heart rate, results of FLASH abdominal ultrasonography (including presence or absence of distended loops of small intestine, thickened intestinal walls, excessive peritoneal fluid, gastric distension, abdominal masses, intestinal intussusception, evidence of colonic displacement and hepatic abnormalities), and volume of any gastric reflux (recorded as spontaneous reflux or reflux obtained on passage of a nasogastric tube). The severity of colic pain was categorised as mild, moderate, or severe, based on a behaviour-based simple descriptive scale (Mair and Smith 2005). Any other significant clinical history or physical examination findings were also recorded. Cases were categorised as medical colic (i.e. colic that resolved with non-surgical treatment), surgical colic / euthanased without surgery (surgical cases euthanased without surgery for financial or welfare reasons), colitis, peritonitis and others. The diagnosis of colitis was based on the typical clinical, ultrasonographic and clinical pathological findings including diarrhoea, tachycardia, fever, leukopenia, hypoproteinemia, etc (Shaw and Stampfli 2018). The diagnosis of peritonitis was based on the presence of an elevated total nucleated cell count and elevated total protein concentration in the peritoneal fluid, with cut-off values for nucleated cell count of $> 10 \times 10^9$/L and total protein concentration > 25 g/L (Brownlow *et al* 1981). The final diagnosis (including non-specific colic) and outcomes (survived, euthanased or died) were recorded.

Serum pancreatic lipase concentrations were recorded and categorised as normal (0-20 U/L), mildly elevated (21–49 U/L), moderately elevated (50– 199 U/L) or markedly elevated (> 200 U/L), as previously described (Johnson *et al* 2019). In addition, pancreatic lipase concentrations that were less or more than twice the upper reference limit (< or >2 x URL) were also recorded (as described by Lanz *et al* 2022). Continuous data were assessed for normality graphically and using Shapiro-Wilk tests. Median, interquartile range (IQR) and range were reported for non-normally distributed data. Categorical data are presented showing the count and percentage. Non-parametric Wilcoxon rank-sum tests and Kruskal-Wallis tests were used for continuous

variables. Post-hoc analysis following a statistically significant Kruskal-Wallis was undertaken using a Dunn's test. Chi-squared tests were used for categorical variables. Correlations between two continuous variables were assessed using Spearman's rank correlation. Statistical significance was set at $p < 0.05$. Data were analysed using Stata 17.0.

Results

Serum pancreatic (DGGR) lipase concentrations were measured in 205 equids. These included 42 ponies, 39 cobs/crosses, 34 sports horses, 26 warmbloods/crosses, 24 Thoroughbred/crosses, 15 Shetland ponies/miniature horses, 12 heavy horses and 13 others (including 3 donkeys). There were 117 geldings, 84 females and 4 entire males. The median age was 13 years (interquartile range [IQR] 9.5; range 0.3-36 years); only 3 horses were younger than 12 months of age. The degree of pain at admission was categorised as mild in 158 animals, moderate in 40 and severe in 3; one animal showed no signs of pain at the time of admission (but was painful at the time that referral was organised). The median heart rate at admission was 52 bpm (IQR 16; range 32-100). The major findings of FLASH ultrasound scans were unremarkable/normal in 114 animals; distended small intestines were recorded in 37, changes compatible with colitis in 16, thickened small intestinal walls in 14, excessive peritoneal fluid in 11, possible colonic displacement in 3, abdominal mass/neoplasia in 3, haemoperitoneum in 2, gastric distension in 2, intussusception in one, and liver abnormality in one (n=204). Gastric reflux (>1 litre) was recorded in 15 horses (range 1-25 litres). The final diagnosis categories were medical colic (n=107), surgical colic/euthanased without surgery (n=53), colitis (n=13), peritonitis (n=15) and other (n=6). The median duration of illness prior to hospitalisation was 12 hours (range 2->100 hours). The outcomes of the cases were survived (n=147), euthanised (n=47) and died (n=11).

The median white blood cell count was 7.2 x 10^9/L (IQR 4.55; range 1.2-35.6 x 10^9/L) (n=185), neutrophil count 5.3 x 10^9/L (IQR 4.2; range 0.4-31.8 x 10^9/L) (n=185), packed cell volume 36.25% (IQR 16.6; range 16.0-86.3) (n=196), serum Amyloid A concentration 13.95 mg/L (IQR 923; range 0-9900 mg/L) (n=186), total plasma protein concentration 60.0 g/L (IQR 10; range 26.0-86.0 g/L) (n=199), plasma albumin concentration 36.5 g/L (IQR 6.25; range 16.0-49.0 g/L) (n=186), plasma lactate concentration 2.0 mmol/l (IQR 19.7; range 0-19.7 mmol/l) (n=144), peritoneal nucleated cell count 1.4 x 10^9/L (IQR 3.9; range 0.3-387.0 x 10^9/L) (n=122) and peritoneal lactate concentration 0 mmol/L (IQR 20.7; range 0-20.7 mmol/L) (n=104). There was a statistically significant association between the systemic lactate concentration and severity of pain ($p = 0.03$). There were statistically significant associations between all of the systemic haematological/biochemical measurements and the type of colic ($p < 0.01$, except WBC ($p = 0.03$)). There were statistically significant associations between peritoneal lactate concentration ($p = 0.01$), blood lactate concentration ($p = 0.02$), albumin concentration ($p < 0.01$) and PCV ($p = 0.02$), and survival of the colic episode.

The median serum pancreatic lipase concentration in all 205 horses was 17 U/L (IQR 14 – 27; range 1 – 3484). Using the methodology described by Johnson *et al* (2019), the lipase concentration was categorised as normal in 124 animals (60.5%) and elevated in 81 (39.5%) (mildly elevated in 55 (26.8%), moderately elevated in 14 (6.8%) and severely elevated in 12 (5.9%)). Serum lipase concentration was less than twice the upper reference limit (<2x URL) in 178 and >2x URL in 27 horses. There was no correlation between age and pancreatic lipase concentration (Spearman's rank: rho = 0.03; $p = 0.68$), and no significant difference between the lipase concentration groups (normal, mildly, moderately, or markedly elevated) and age (Kruskal-Wallis test; $p = 0.93$), or between horses with lipase concentration < or > 2xURL and age (Kruskal-Wallis tests: $p = 0.62$). There was no significant difference in breed and lipase concentration (Kruskal-Wallis tests $p = 0.33$ for lipase categories, and Chi-Squared p=0.39 for < or > 2xURL), or in sex and lipase concentration (Kruskal-Wallis tests $p = 0.52$ for lipase categories, and Chi-squared p=0.33 for < or > 2xURL). There were no significant associations between any of the haematological or biochemical findings and the lipase concentration groups.

There was a statistically significant difference in the disease category and pancreatic lipase concentration categories (Kruskal-Wallis test: $p < 0.001$) as well as a significant difference between the disease category

and lipase concentration < or > 2xURL (Chi-squared p<0.01) , with colic cases having higher lipase concentrations than colitis and peritonitis cases: medical colics, n = 107, median lipase = 17 U/L (IQR 14 – 30); surgical colics /euthanased, n = 53, median lipase = 21 U/L (IQR 16 – 32); colitis, n = 24, median lipase = 13 U/L (IQR 11 – 17); peritonitis, n = 15, median lipase = 15 U/L (IQR 13 – 18); others, n = 6 median lipase = 84 U/L (IQR 28 – 1094). There were statistically significant differences in lipase concentrations between these disease categories (Dunn's post-hoc test) (Figure 1). Significant differences were present between medical colic and colitis cases (p < 0.001), surgical colic and colitis cases (P < 0.001), and surgical colic and peritonitis cases (p = 0.02). There was no significant association between heart rate and lipase concentration group (Kruskal-Wallis: p = 0.80) or between heart rate and lipase concentration < or > 2xURL (Kruskal-Wallis p=0.24), and no correlation between heart rate and lipase value (Spearman's rank: rho = 0.09; p = 0.21). There was strong evidence of an association between pain severity and lipase values (Kruskal-Wallis: p = 0.01) and between pain severity and lipase concentration < or > 2xURL (Chi-squared p=0.05), with higher lipase concentrations in horses with more severe pain: mild pain, n = 158, median lipase = 17 U/L(IQR 14 – 25); moderate pain, n = 40, median lipase = 19 U/L (IQR 15 – 47); severe pain, n = 4, median lipase = 558 U/L (IQR 22 – 2186). There was no evidence of an association between outcome (classified as survived, euthanasia or died) and lipase concentration (Kruskal-Wallis: p = 0.36), however there was a difference in outcome across lipase concentration groups categorised as < or > 2xURL (Chi-squared p=0.04). There was no difference in duration of colic between lipase concentration groups (Kruskal-Wallis: p = 0.10) or lipase concentration categorised as < or > 2x URL (Chi-squared p = 0.63).).

Differences were identified in pancreatic lipase concentration category (Dunn's post hoc test) for the following FLASH ultrasound findings: no abnormalities vs colitis, p < 0.001; distended small intestines vs colitis, p < 0.001; colitis vs colonic displacement, p = 0.05. The median pancreatic lipase concentration of horses with different findings on FLASH ultrasonography were as follows: horses with no significant abnormalities on FLASH ultrasonography (n=114) had a median pancreatic lipase concentration of 19 U\L (IQR 14 – 32) (98 horses had lipase < 2xURL and 16 horses > 2xURL); median lipase concentration of horses with colitis (n = 16) was 12 U/L (IQR 10 – 13) (16 horses had lipase concentration < 2xURL and none with lipase concentration > 2xURL); median lipase concentration of horses with distended small intestines (n = 37) was 19 U\L (IQR 15 – 32) (29 horses had lipase concentration < 2xURL and 8 had lipase concentration > 2xURL); median lipase concentration of horses diagnosed with a potential colonic displacement on FLASH ultrasound (n = 3) was 25 U\L (IQR 22 – 36) (all 3 horses had lipase concentration < 2xURL). Of the 12 horses with severely increased pancreatic lipase concentration on admission, 3/12 (25.0%) had spontaneous nasogastric reflux (spontaneous nasogastric reflux was not recorded in any of the other 193 equids), 6/10 (60.0%) had distended and/or thickened small intestine on abdominal ultrasonography (not performed in 2 cases), 7/12 (58.3%) survived to hospital discharge, and 5/12 (41.7%) died or were euthanised (in 2 cases surgery was recommended but the owners opted for euthanasia for financial reasons). Overall, 15/205 equids (7.3%) had reflux (> 1 litre) either spontaneously or on passage of a nasogastric tube recorded at admission. Four of these (26.7%) had normal pancreatic lipase concentrations, 6 (40.0%) had mildly elevated concentrations and 5 (33.3%) had severely elevated concentrations. In total, therefore, 11 of 15 (73%) horses that had gastric reflux identified at admission to the hospital had elevated serum pancreatic lipase concentrations. 21 of 51 horses (41%) where FLASH scanning identified distended small intestines or thickened small intestinal walls had elevated serum pancreatic lipase concentrations (12 mildly elevated, 3 moderately elevated and 6 severely elevated). The final diagnoses in 10 animals with severely increased serum pancreatic lipase concentrations where a diagnosis was reached included non-specific colic (n=4), enteritis (n=2), grain overload (n=1), post-foaling colic and neuropathy (n=1), epiploic foramen entrapment (n=1) and colon torsion (n=1).

Repeated analyses for pancreatic lipase were performed in 15 animals (Table 1). In 11 of these horses that had a serum pancreatic lipase concentration > 21 U/L on admission to the hospital, the concentration reduced in subsequent samples (following surgical or medical treatment and resolution of the abdominal pain).

Discussion

Our results indicated that many equids with gastrointestinal disease/colic have increased serum pancreatic (DGGR) lipase concentrations. The results showed a significantly higher median lipase activity in horses with colic than those with colitis or peritonitis, suggesting that a degree of pancreatitis may be present in many colic cases, although this does not indicate causation, and, in many cases, this is likely to be a secondary response, as is noted in people and other species (Watson *et al* 2010; Watson 2015). An increased risk of pancreatitis, as determined by increased serum pancreatic lipase concentrations, has previously been reported in colic (Johnson *et al* 2019; Lanz *et al* 2022). However, increased pancreatic lipase activity has also been reported in animals, including horses, with a variety of other non-gastrointestinal disease. For example, in the study of Johnson *et al* (2019), hyperlipaseamia was identified in 16 horses affected by various conditions, including poor athletic performance, lameness, anaemia, rhabdomyolysis, laminitis, cellulitis, juvenile osteoarthritis and training. However, in the current study, DGGR lipase concentrations were significantly higher in colic cases compared to colitis and peritonitis cases, providing further evidence to suggest a possible association between colic and pancreatitis. In addition to this apparent association with colic, there were significant associations between pain severity and lipase values in this study, with higher lipase activities in horses with more severe pain. However there was no significant association with heart rate. Although heart rate is to some extent dependent on the level of pain and sympathetic response, the rate is also significantly affected by other factors including vascular volume and cardiovascular status (including hypovolaemia and systemic inflammatory syndrome), which may explain why there was an observed association with pain but not with heart rate. We also found no association between pancreatic lipase activities and breed in this study; this differs from the results of Johnson *et al* (2019) who found that hot-blooded horses were more frequently affected.

Johnson *et al* (2019), reported increased DGGR-lipase activity above the upper reference limit of 20 U/L in 40% of 109 hospitalised horses; 33 of these horses had gastrointestinal disease, of which 28 (85%) had increased pancreatic lipase activity. This compares with 39.5% of equids with gastrointestinal disease that had hyperlipaseaemia in the current study. Lanz *et al* (2022) measured serum DGGR-lipase activity in a larger number (192) of horses with colic; increased DGGR-lipase activity was demonstrated in 30.2% of horses with signs of colic and was above twice the upper reference limit in 15.6%. These results more closely align with the results of our study, where pancreatic lipase concentration $>$ 2xURL was identified in 13.2% of equids with gastrointestinal disease. The marked differences in the prevalence of hyperlipaseaemia in the study of Johnson *et al* (2020) with that of Lanz *et al* (2022) and our own study could represent differences in the populations assessed; in the Johnson*et al* (2019) study there were larger numbers of foals (up to a year) and Thoroughbreds in training. Compared with horses with normal pancreatic lipase concentrations, horses with colic with hyperlipaseaemia in the study of Lanz *et al* (2022) had higher percentages of non-survivors, surgical cases, strangulating diseases, and large bowel displacements or torsion. In the current study we were unable to demonstrate a statistically significant association between pancreatic lipase concentration and outcome or any specific type of disease, apart from identifying significant differences between medical colic and colitis cases, surgical colic and colitis cases, and surgical colic and peritonitis cases. This could also be reflective of a difference in the population of animals studied. In our study, we also found a significant difference in outcome (survival, euthanasia, died) and lipase concentrations $<$ or $>$ 2xURL. One other study (Bartel *et al* 2023) reported an elevation of pancreatic lipase concentration in one horse with colic (out of a total of 19 horses tested). It is noteworthy that the prevalence of increased DGGR lipase concentrations in horses with signs of colic in all of these studies was higher than the prevalence of primary pancreatitis reported in necropsy studies (Yamout *et al* 2012; Newman, 2015). This finding suggests that hyperlipaseaemia, as diagnosed by increased DGGR lipase activity, probably results from secondary pancreatic damage in many cases (Lanz *et al* 2022).

Assessing the relevance of increased pancreatic lipase concentrations in this and other studies is hampered by the lack of histopathological examination of the pancreas; this was not feasible in the current study. The prevalence of pancreatitis, based on the presence of hyperlipaseaemia in this and other studies, is much higher than that identified previously, before the DGGR lipase assay was available. For example, the presence of pancreatitis, based on gross post-mortem and histological examination, was diagnosed in only 4/834

(0.4%) horses necropsied over nine years in one study (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 agreement with the current and more recent studies where pancreatitis was diagnosed using serum DGGR-lipase activity (Johnson *et al* 2019; Lanz *et al* 2020), the study by Yamout *et al* (2012) showed that pancreatitis occurred frequently in association with gastrointestinal disease, including large bowel torsion, colonic displacements, colonic impaction, enteritis, small intestinal strangulation, etc. Pancreatitis associated with the latter diseases has been reported previously. We also diagnosed pancreatitis in a horse with entrapment of jejunum in the epiploic foramen and it seems likely that trauma to the pancreas might be the cause in such cases, since the pancreas borders the epiploic foramen; an association with this disease has been recorded previously (Johnson*et al* 2019). Displacement and torsion of the large colon has also been associated with pancreatitis in several previous studies, possibly due to disruption of the blood supply to the pancreas (Yamout *et al* 2012) or shock-related hypoperfusion and mechanical compression (Grulke *et al* 2003).

In dogs, increased pancreatic lipase activity may be also observed in a variety of other non- pancreatic diseases, including portal hypertension, gastrointestinal, renal, and endocrine disorders (Rallis*et al* 1996; Prummer *et al* 2020; Serrano *et al* 2021); in the present study, increased DGGR-lipase concentration was identified secondary to several other conditions, including non-specific colic, enteritis, grain overload and post-foaling colic. Acute pancreatitis has previously been reported in a donkey with grain overload (Kawaguchi *et al* 2004).

Spontaneous gastric reflux in association with severely elevated DGGR lipase activity was identified in 3 horses is this study, and gastric reflux on passage of a nasogastric tube was present in a further 8 horses with severe hyperlipaseaemia; in total 11 of 15 (73%) horses with severely raised pancreatic lipase concentration had gastric reflux. This association has been recorded previously (Buote 2003; Kawaguchi et 2004; Waitt *et al* 2006; Bakos *et al* 2008; Yamout *et al* 2012; Newman 2015; Lohmann and Allen 2015). The presence of gastric reflux in a horse showing signs of acute colic should, therefore, raise suspicion of pancreatitis if no other reason for the gastric reflux (such as small intestinal obstruction) can be identified. Likewise, the identification of distended loops of small intestine or thickened small intestinal walls of FLASH scanning could indicate possible pancreatitis if no other cause of these findings can be recognised. Distended loops of small intestine have been identified either on rectal palpation or ultrasonographically in cases of acute pancreatitis previously (Buote 2003; Kawaguchi *et al* 2004; Bakos *et al* 2008; Schmidt*et al* 2010; Yamout *et al* 2012; Newman 2015; Edery *et al* 2015; Gomez *et al* 2015), however this finding is commonly seen in horses with primary diseases causing small intestinal obstruction (Beccati *et al* 2011).

We were unable to visualise the pancreas on abdominal ultrasonography. Ultrasonographic identification of pancreatic damage, including a mass effect between the liver and right dorsal colon has been previously described in a 24-year-old Warmblood gelding with recurrent colic (Lack*et al* 2021). However, detailed ultrasonographic examination of this area is not a specific aim of the FLASH procedure which was undertaken in animals in this study.

In most animals which had elevated serum pancreatic lipase concentrations at admission, and where sequential assays were undertaken, the DDGR lipase concentrations reduced as the clinical condition improved. This has been reported previously in two horses with right dorsal displacement of the colon (Johnson *et al* 2019). However, in the latter study, one of the horses showed a recurrence of pain and a subsequent elevation of plasma DGGR lipase concentration 8 days after the initial episode of colic, suggesting that some horses may be prone to recurrent episodes of pancreatitis, although this might reflect recurrence of the gastrointestinal disease rather than recurrence of primary pancreatitis.

There are several limitations of this study. We were unable to confirm the presence of pancreatitis in any of the horses with elevated serum DGGR lipase concentrations by post-mortem examination or histopathology. This histopathological confirmation has not been reported in any other studies, and requires further investigation. Traditionally, histopathology has been considered to be the gold standard for the diagnosis of pancreatitis and for distinguishing acute pancreatitis from chronic pancreatitis (Cridge *et al* 2021). However, the histopathological appearance does not always reflect the clinical presentation of affected patients, and

reliance on a histopathologic diagnosis for acute pancreatitis is considered to be questionable because lesions can be highly localised and the immediate clinical relevance of lesions is unclear (Mansfield *et al* 2012). The work of Johnson *et al* (2019) indicates that DGGR lipase is highly specific for the equine pancreas, and it is widely used as a marker of pancreatitis in other species (O'Brien *et al* 2014; Cridge*et al* 2021). In addition, similar to previous reports of the use of DGGR lipase in equids (Johnson *et al* 2019; Lanz *et al* 2020), the sample size for this study was small. Future studies incorporating larger numbers and correlation with specific diagnostic imaging and post-mortem/histopathological findings are warranted. The results of this study should be appraised with reference to the population of animals examined. In this study, all of the animals were privately owned, and most commonly used for pleasure, so the results cannot necessarily be extrapolated to other patient groups such as competition horses and racehorses. A key disadvantage of convenience sampling that was used in this study is that the sample lacks clear generalizability. We were reliant on the availability of surplus blood samples, and there was a likely bias towards sampling surgical colics, colitis cases and peritonitis cases because these cases would be more likely to have surplus blood samples available.

In conclusion, the results of this study indicate that pancreatitis, as evidenced by increased serum DGGR lipase concentrations, is frequently present in horses presenting with signs of colic. In many cases this seems likely to represent a secondary pancreatitis. This observation appears to be more common in certain diseases including colon torsion, colonic displacements, enteritis, entrapment of the small intestine in the epiploic foramen, etc. In addition, horses with colic presenting with gastric reflux (including spontaneous reflux) and horses with ultrasonographic evidence of distended/thickened loops of small intestine may be more likely to have elevated DGGR lipase concentrations. In most cases, the DGGR lipase concentration reduces once the underlying disease resolves. However, in some cases, it is possible that primary acute pancreatitis may cause colic signs, although distinguishing these from secondary pancreatitis associated with gastrointestinal disease may be challenging.

Legends

Figure 1. Differences in serum DGGR lipase activities between disease categories (Dunn's post-hoc test)

Acknowledgements

The authors thank Michelle Moreton-Clack and colleagues at Bell Equine Veterinary Clinic for assistance with collection of blood samples and laboratory analyses.

This study was supported by funding from a CVS Group Clinical Research Grant.

References

Bakos, Z., Krajcsovics, L. and Toth , J. (2008) Successful medical treatment of acute pancreatitis in a horse. *Vet.Rec.* **162** 95-96

Bartel, H., Rijkenhuizen, A. B. M., Sommerey, C. C., Stock, G. and Shell, K. (2023) Occurrence of elevated amylose and lipase enzymes in horses with gastrointestinal disease. *Pferdeheilkunde* **39** 515-522

Beccati, F., Pepe, M., Gialletti, R., Cercone, M., Bazzica, C. and Nannarone, S. (2011) Is there a statistical correlation between ultra- sonographic findings and definitive diagnosis in horses with acute abdominal pain? *Equine Vet.J.* **Suppl 39** 98–105

Bjørnkjær-Nielsen, K-A and Bjørnvad, C.R. (2021) Corticosteroid treatment for acute/ acute-on-chronic experimental and naturally occurring pancreatitis in several species: a scoping review to inform possible use in dogs. *Acta Vet.Scand.* **63** 28 https://doi.org/10.1186/s13028-021-00592-0

Brownlow, M.A., Hutchins, D.R. and Johnston, K.G. (1981) Reference values for equine peritoneal fluid. *Equine Vet.J.* **13** 127–130

Buote, M. (2003) Cholangiohepatitis and pancreatitis secondary to severe gastroduodenal ulceration in a foal. *Can.Vet.J.* **44** 746–748

Cridge, H,. Twedt, D.C., Marolf, A.J., Sharkey, L.C. and Steiner, J.M. (2021) Advances in the diagnosis of acute pancreatitis in dogs. J.*Vet.Intern.Med.* **35** 2572-2587

Edery, N., Rosenbaum, A., Busnach, A., Steinman, A., Tirosh Levy, S. and Perl, S. (2015) Acute Pancreatitis in a Horse – a Case Report.*Israel J.Vet.Med.* **70** 49-52

Gomez, D.E., Radtke, C.L., Russell, L.A., Lopez, A. and Wichtel, M.W. (2015) Acute pancreatitis following granulosa cell tumor removal in a mare. *Can.Vet.J.* **56** 1049–1052

Grulke, S., Gangl, M., Deby-Dupont, G., Caudron, I., Deby, C. and Serteyn, D. (2003) Plasma trypsin level in horses suffering from acute intestinal obstruction. *Vet.J.* **63** 283–291

Harris, V.D. and Gow, W.J. (1892) Ferment actions of the pancreas in different animals. *J.Physiol.* **13** 469–492

Johnson, J.P., Stack, J.D, McGivney, C.L., Garrett, M.P. and O'Brien, P.J. (2019) DGGR-lipase for effective diagnosis of pancreatitis in horses. *Comp.Clin.Pathol.* **28** 1625–1636

Kawaguchi, K., Church, S. and Slocombe, R.F. (2004) Acute necrotising pancreatitis following grain overload in a donkey.*Aust.Vet.J.* **82** 619-621

Lack, A.C., Crabtree, N.E. and Slocombe, R.F. (2021) Clinical pathology and ultrasonographic !ndings from a Warmblood gelding with primary, severe, acute pancreatitis. *Equine Vet.Educ.* **33** e303-e308

Lanz, S., Howard, J., Gerber, V. and Peters, L. M. (2022) Diagnostic utility and validity of 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase activity in horses with colic. Vet.J. 288 105887

Lohmann, K.L. and Allen, A.L. (2015) Chronic active interstitial pancreatitis as a cause of transverse colonic obstruction and colic in a horse. *Can.Vet.J.* **56** 1177–1180

Lorenzo-Figueras, M., Morisset, S., Morisset, J., Lainé, J. and Merritt, A.M. (2007) Digestive enzyme concentrations and activities in healthy pancreatic tissue of horses. *Am.J.Vet.Res.* **68** 1070–1072

Mair, T.S. and Smith, L.J. (2005) Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 1: short-term survival following a single laparotomy. *Equine Vet.J* . **37** 296-302

Mansfield, C. (2012a) Acute pancreatitis in dogs: advances in understanding, diagnostics, and treatment. *Top.Companion Anim.Med.* **27** 123–132

Mansfield, C. (2012b) Pathophysiology of acute pancreatitis: Potential application from experimental models and human medicine to dogs.*J.Vet.Intern.Med.* 26 875–887

Mansfield, C.S., Anderson, G.A. and O'Hara, A.J. (2012) Association between canine pancreatic-specific lipase and histologic exocrine pancreatic inflammation in dogs: assessing specificity. *J.Vet.Diagn.Investig* . **24** 312-318

Newman, S.J. (2015) Equine pancreatic disease: a review and characterization of the lesions of four cases (2005-2014).*J.Vet.Diagn.Invest.* **27** 92–96

O'Brien, P.J., Papakonstantinou, S. and McGrath, C. (2014) Pancreatic, DGGR- lipase, enzymatic assay is a highly sensitive, specific and inexpensive biomarker of pancreatitis in cats and dogs. *Vet.Clin.Pathol.* **42** E20–E48

Peters, L.M. and Howard, J. (2023) Dataset of partial analytical validation of the 1,2-0-dilauryl rac-glycero-3-glutaric acid-(6-Methylresorufin) ester (DDGR) lipase assay in equine plasma.*Data* **8** 50 doi.org/10.3390/data8030050

Prummer, 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. *J.Vet.Int.Med.* **34** 2319–2329

Rallis, T.S., Koutinas, A.F., Kritsepi, M. and Moraitou, K.T. (1996) Serum lipase activity in young dogs with acute enteritis or gastroenteritis. *Vet.Clin.Pathol.* **25** 65–68

Schmidt, B., Kappe, A., Spallek, A., Uhlig, A., Schoon, H. A., Coenen, M. and Schusser, G. F. (2010) Acute necrotising pancreatitis as colic reason in a pony. *Pferdeheilkunde* **26** 775-780

Serrano, G., Paepe, D., Williams, T. and 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. *J.Vet.Diag.Invest.* **33** 548–553

Shaw, S.D. and Stämpfli ,H. (2018) Diagnosis and treatment of undifferentiated and infectious acute diarrhea in the adult horse. *Vet.Clin.North Am.Equine Pract* . **34** 39-53

Talukdar, R., Sareen, A., Zhu, H., Yuan, Z., Dixit, A., Cheema, H,. George, J,. Barlass, U,. Sah, R., Garg, S.K., Banerjee, S., Garg, P., Dudeja, V., Dawra, R. and Saluja, A.K. (2016) Release of cathepsin B in cytosol causes cell death in acute pancreatitis. *Gastroenterol.* **151** 747–758

Tietz, N.W. and Shuey, F.S. (1993) Lipase in serum - the elusive enzyme: an overview. *Clin.Chem.* 1993 **39** 746–756

Waitt, L.H., Cebra, C.K., Susan J. Tornquist, SJ. and Lo hr, C.V. (2006) Panniculitis in a horse with peripancreatitis and pancreatic fibrosis. *J.Vet.Diagn.Invest.* **18** 405–408

Watson P. (2015) Pancreatitis in dogs and cats: definitions and pathophysiology. *J.Small Anim.Pract.* **56** 3–12

Watson, P.J., Archer, J,. Roulois, A.J., Scase, T.J, and Herrtage, M.E. (2010) Observational study of 14 cases of chronic pancreatitis in dogs. *Vet.Rec.* **167** 968–976

Xenoulis, P.G. (2015) Diagnosis of pancreatitis in dogs and cats. *J.Small Anim.Pract* . **56** 13-26

Yamout, S.Z., Nieto, J.E., Anderson, J., Decock, H.E.V., Vapniarsky, N. and Aleman, M. (2012) Pathological evidence of pancreatitis in 43 horses (1986–2011) *Equine Vet.J.* **44 Suppl. 43** 45–50

Acknowledgements

This study was supported by a research grant from CVS Group. We thank Michelle Moreton for her assistance in laboratory testing of the blood samples.

Table 1. Results of repeated serum pancreatic lipase assays in 15 horses with gastrointestinal disease.

Case	Lipase Concentration u/L								Diagnosis
	Day of admission = Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
1	22	15							Entrapment of jejunum
2	61				16				Colitis
3	15			14					Small intestinal strangu
4	40				15				Non-specific colic
5	87		33						Right dorsal displacem
6	2616		87						Non-specific colic
7	17		18						Non-specific colic
8	1770		28						Non-specific colic
9	105		13						Pelvic flexure impactio
10	16		38		21				Pelvic flexure impactio
11	31			19					Pelvic flexure impactio
12	445			19					Enteritis

Case	Lipase Concentration u/L	Diagnosis						
13	26		14					Small intestine strangu
14	60		13					Non-specific colic
15	215	208	1515	1563	460	130	52	Non-specific colic