Pferdeheilkunde – Equine Medicine 39 (2023) 6 (November/December) 515–522 DOI 10.21836/PEM20230602

Occurrence of elevated amylase and lipase enzymes in horses with gastrointestinal disease

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Summary: Pancreatic disease in horses is rare and diagnosis is difficult due to lack of available diagnostic tests. Acute pancreatitis is associated with acute colic signs and gastrointestinal reflux; chronic pancreatitis with weight loss, anorexia, lethargy, and mild recurrent colic symptoms. Therefore, horses presented with such unspecific symptoms are often treated routinely and a concurrent pancreatic disorder may be missed. A prospective study was initiated to investigate the occurrence of pancreatic disease in horses with corresponding clinical signs. During the study period from 2018–2019, sixty-seven horses (39 geldings, 26 mares, 2 stallions) aged 1–26 years with Warmbloods being the predominant breed were included. In 48 horses, the pancreatic enzymes α-amylase (AMY) and DGGR-lipase (LIP) were measured in serum (S) and peritoneal fluid (PF). In addition, cytologic examination from PF, complete blood count (CBC) and serum biochemistry (SBC) were performed. In 19 horses, including 14 control horses without clinical signs, pancreatic enzymes were measured from serum samples only. In one horse with colic symptoms, LIP was elevated in S; in another horse with duodenitis-proximal jejunitis and septic peritonitis, LIP was elevated in PF. In both horses, a pancreatic component was considered to play a role. A third horse had measured enzymes within normal ranges, but postmortem examination revealed chronic pancreatitis. These results suggest that pancreatic disease has a low prevalence in horses, but should be considered as an additional differential diagnosis in patients with indistinct abdominal symptoms.

Keywords: pancreatitis, horse, amylase, lipase, colic symptoms

Citation: Bartel H., Rijkenhuizen A. B. M., Sommerey C.-C., Stock G., Shell K. (2023) Occurrence of elevated amylase and lipase enzymes in horses with gastrointestinal disease. Pferdeheilkunde 39, 515–522; DOI 10.21836/PEM20230602

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Submitted: Juli 18, 2023 | **Accepted:** September 24, 2023

Introduction

Pancreatic disease has been described for decades as a rare disorder in equids and is diagnosed mostly at necropsy due to lack of available and reliable antemortem diagnostic tests (*Higginson* 1937, *Talbot* et al. 2011, *Yamout* et al. 2012, *Edery* et al. 2015, *Newman* 2015). Both primary and secondary pancreatitis can occur. Primary pancreatitis is defined as pancreatic lesions as the main or only pathological finding, whereas secondary pancreatitis occurs as a result of any other underlying disorder (*Yamout* et al. *2012*).

Postmortem findings include acute, chronic and chronic active pancreatitis (*Breider* et al. 1985, *Taintor* et al. 2006, *Bakos* et al. 2008, *Talbot* et al. 2011, *Ollivett* et al. 2012, *Yamout* et al. 2012, *Newman* 2015) and neoplasia (*Ross* et al. 1983, *Church* et al. 1987, *Carrick* et al. 1992, *Rendle* et al. 2006, *Barsnick* et al. 2008, *Spanton* et al. 2009, *Brot* et al. 2014, *Herbach* et al. 2014).

Diagnosing pancreatitis remains challenging. Whereas acute pancreatitis in adult horses is characterized by severe abdominal pain, ileus with duodenogastric reflux and hypovolemia (*Bakos* et al. 2008, *Schmidt* et al. 2010, *Yamout* et al. 2012, *Newman* 2015, *Lack* et al. 2020), chronic pancreatitis presents with weight loss, lethargy, mild recurrent colic and inappetence (*Breider* et al. 1985, *Yamout* et al. 2012, *Leipig* et al. 2015). Coma and cerebral dysfunction as predominant clinical findings have been reported in two cases of severe acute pancreatitis in foals (*Taintor* et al. 2006, *Ollivett* et al. 2012). Currently there is no standardized test to diagnose pancreatitis in horses. Elevated serum enzyme activities of amylase (AMY) and lipase (LIP), secreted by the acinar cells of the pancreas, are considered useful to diagnose acute pancreatic disease in horses, although they are of limited diagnostic accuracy.

For diagnosing acute pancreatitis in humans, measurement of LIP is preferred because it is tissue-specific and has a sensitivity between 64% and 100%, whereas sensitivity of AMY for diagnosis of acute pancreatitis is between 45% and 87% (*Ismail* and *Bhayana* 2017). The specificities for both enzymes range between 92% and 99%. LIP has a larger time slot than AMY, as it rises 3–6 hours after onset of clinical signs and remains elevated for up to two weeks, whereas AMY peaks rapidly after the onset of clinical signs, has a short half-life of 10–12 hours and returns to normal within three to five days (*Ismail* and *Bhayana* 2017).

In recent research, the 1′2-o-dilauryl-rac-glycero-3-glutarate- (6′-methylresorufin) ester (DGGR) lipase assay has been determined to be a pancreas-specific parameter in horses (*Johnson* et al. 2019). Determination of DGGR-LIP (LIP) and AMY in horses is expected to be of diagnostic value for pancreatic disease, although AMY has limitations as a single assay. AMY activity in the pancreas is low (*Lorenzo-Figueras* et al. 2007, *Johnson* et al. 2019) and activity of AMY is a one-half LIP half-life period in plasma (*Johnson* et al. 2019). Besides, hyperamylasemia can also occur in intestinal and renal disorders (*Clink* et al. 1982), which are more common than pancreatic disease. This preliminary research aimed to investigate the occurrence of elevated pancreatic enzymes (AMY and LIP) in horses with gastrointestinal disorders in serum (S) and peritoneal fluid (PF). The objective was to determine if measurement of pancreatic enzymes can be helpful as a screening tool to identify horses with pancreatic disease ante mortem and to document clinical cases. The study hypothesis was that pancreatic disease occurs in horses with clinical signs of gastrointestinal disease.

Material and methods

The study was performed from 2018 to 2019 and included 67 horses (39 geldings, 26 mares, 2 stallions) aged 1–26 years (median age 13 years) with Warmbloods being the predominant breed (50.7%), divided into two groups. Group A included 53 horses, which were presented for diagnostic workup of gastrointestinal and hepatic disease with one or more of the following symptoms: acute colic signs with and without duodenogastric reflux, peritonitis, weight loss, anorexia, lethargy, mild recurrent colic symptoms and elevated liver enzymes. The pancreatic enzymes AMY and LIP were measured in S and PF samples taken at admission. Unfortunately, in five horses no peritoneal fluid could be obtained. Complete blood count (CBC) and serum biochemistry (SBC) were performed as point-of-care examinations (IDEXX ProCyte, IDEXX Catalyst One). Cytology from PF was performed at the IDEXX reference laboratory. Group B included 14 clinically healthy horses as negative control group, presenting for orthopedic complaints without signs of gastrointestinal disease (5 geldings, 8 mares, 1 stallion, median age 12.5 years). In these horses, routine blood work was performed, and available samples served for measurement of AMY and LIP. For this study, institutional animal care and use committee approval was not required as residual material from routine diagnostic work-up was used. Owner consent was obtained for use of remaining samples for research purposes.

Blood samples were centrifuged and serum was refrigerated at 4 °C. Peritoneal fluid samples were smeared immediately after sampling and the remainder equally refrigerated at 4 °C until handling in the laboratory. All samples were sent overnight and analyzed at the IDEXX reference laboratory the following day and results were available 1–2 days after sending.

Enzymes were measured by photometry, for lipase an enzymatic colorimetric assay with 6-methyl-resorufin ester as substrate (Roche; Beckman Coulter AU680 clinical chemistry analyzer) was performed. Alpha amylase was measured by a kinetic colorimetric assay with ethylidene-G7PNP as substrate (Beckman Coulter; Beckman Coulter AU680 clinical chemistry analyzer). Reference values for LIP (laboratory specific internal validation) were serum activity < 250 international units per litre (IU/L) and 0–400 IU/L for AMY (Kraft Dürr, Klinische Labordiagnostik, 5. Auflage, Schattauer Verlag, 1999). For PF, laboratory-specific reference ranges were not available. Cytologic examinations of PF were performed of direct smears and after cytospin, May-Grünwald-Giemsa staining was conducted (IDEXX GmbH).

Results

In group A, mean S AMY was 14.5 IU/L, S LIP 27 IU/L, PF AMY 12.9 IU/L, and PF LIP 27.44 IU/L. Based on the enzyme activities, two horses were suspected to have a pancreatic disease.

Details of these two cases are described in detail, as well as a third horse, which had normal enzyme activities but post-mortem diagnosis of chronic pancreatitis. In group B, serum enzymes were within the reference ranges in all cases, mean S AMY was 10.8 IU/L, mean S LIP was 13 IU/L.

Clinical cases

Case 1

Case 1 was a 20-year-old Appaloosa mare, presenting with colic symptoms of one day duration. Treatment by the referring veterinarian consisted of N-butylscopolamine (0.2 mg/kg per bodyweight [bwt] intravenously [IV], metamizol-natrium 25 mg/kg bwt IV, Buscopan comp.®)[a] and butorphanol (Torbugesic®Vet, 0.01 mg/kg bwt IV)[b]. Two months before presentation the horse underwent celiotomy due to large colon displacement in another hospital.

On arrival, the horse showed mild signs of abdominal discomfort, vital parameters were within normal ranges. Nasogastric intubation revealed no net reflux, feed was obtained during flushing. Transrectal palpation and transabdominal ultrasound examination (5–2 MHz-probe, Sonosite®)[c] were unremarkable.

CBC showed a mild hypochromic anemia (packed cell volume [PCV] 0.23 L/L, reference range [rr] 0.32–0.40 L/L). Red blood cells (RBC) were decreased (5.07 T/L, rr 6.4–10.4 T/L), haemoglobin was 8.8 mmol/L (rr 10.7–16.5 mmol/L).

Abdominocentesis revealed clear peritoneal fluid with a normal total nucleated cell count ([TNCC] 3.4×10^9/L, rr $< 5.0 \times 10^9$/L), lactate (0.89 mmol/L, rr < 1.78 mmol/L), and total protein ([TP] 12 g/L, rr < 25 g/L). Cytology of the abdominal fluid was unremarkable.

Another period of mild colic three hours later resolved after administration of N-butylscopolamine (0.2 mg/kg bwt IV), and metamizol-natrium (25 mg/kg bwt IV, Buscopan comp®)[a].

Gastroscopy (gastroscope, 320 cm, STORZ®)[d] was performed the following day. The stomach was still filled with gastric content despite fasting for 14 hours. After treatment with metoclopramide (0.25 mg/kg IM every 6 hours, Metomotyl®)[e], the stomach was completely empty the next day and the gastric

mucosa was unremarkable in all aspects. The horse was re-fed and did not show clinical signs of discomfort again. Diagnosis was spasmodic colic with secondary gastric dilation and repeated CBC due to mild anemia was recommended. The owner reported that the mare was doing well a few days after discharge.

Laboratory results of AMY, LIP and cytology arrived after discharge of the horse. Serum AMY was within the reference range measured 189 IU/L, S LIP was 489 IU/L (rr < 250 IU/L). In PF, AMY was 39 IU/L and LIP measured 105 IU/L.

The elevated LIP activity in S was suggestive of concurrent pancreatitis. In PF, LIP activity was also high in comparison to reference ranges of S, as there are no laboratory specific reference ranges for PF.

Cytology of the abdominal fluid was unremarkable.

Case 2

A 9-year-old Haflinger gelding presented due to acute colic symptoms for a few hours and suspected small intestinal obstruction. The horse was referred after administration of flunixine-meglumine (1.1 mg/kg bwt IV, Flunidol®)[f] and obtaining 10 L of duodenal reflux into the stomach by nasogastric intubation.

On arrival, the horse was reduced in behaviour, tachycardic (heart rate 56 beats per minute [bpm]) and showed mild signs of colic. Capillary refill time was prolonged and nasogastric intubation revealed 5 L of duodenogastric reflux. Routine blood work showed mild haemoconcentration (PCV 0.38 L/L, rr 0.32–0.40 L/L) with hyperproteinemia (87 g/L, rr 56–79 g/L) and hyperalbuminemia (34 g/L, rr 19–32 g/L), neutrophilic leucocytosis with left shift (WBC 12.97 G/L, rr 4.9–11.1 G/L), elevation of Alkaline Phosphatase ([ALKP] 829 U/L, rr 10–326 U/L), elevated ɣ-Glutamyltransferase ([GGT] 181 U/L, rr 0–87 U/L), increased globulin (53 g/L, rr 24–47 g/L) and hyperglycemia (9.05 mmol/L, rr 3.55–8.32 mmol/L). Mild colon impaction of the flexura pelvina was palpated transrectally, transabdominal ultrasound examination showed increased free abdominal fluid and a few distended small intestinal loops. Peritoneal fluid obtained by abdominocentesis was turbid with an elevated TNCC 21.77×10^9/L (rr < 5.0×10^9/L) and elevated lactate of 5.1 mmol/L (rr < 1.78 mmol/L).

Due to severe signs of colic, diagnostic laparotomy via ventral midline incision was performed immediately and enteritis was diagnosed. The amount of peritoneal fluid was increased and turbid. The flexura pelvina of the colon ascendens was impacted and slightly displaced to the right side, the caecum was displaced caudally. The stomach was distended with gas, the jejunum was gas-distended and showed marked hypomotility, the ileum wall was thickened. An enteritis was suspected on base of the findings during surgery. Full-thickness biopsies from jejunum and ileum showed marked oedema of the intestinal wall at histopathologic examination without signs of inflammation.

Serum AMY (< 10 IU/L) and LIP (80 IU/L) were within normal ranges. In PF, both enzymes were elevated compared to serum values, AMY was 342 IU/L and LIP measured 706 IU/L. Cytology revealed an inflammatory peritoneal effusion of increased cellularity with a high amount of partially degenerated neutrophil granulocytes. Bacterial culture growth of *E. coli ssp*. with haemolysis was present. Diagnosis of septic peritonitis was made. Based on the high activities of PF AMY and LIP concurrent pancreatitis was suspected. Cytology results and enzyme activities were available one day after surgery.

Postoperative treatment consisted of lidocaine (Lidocainhydrochloride 2%®)[g] as constant-rate infusion ([CRI], 0.05 mg/kg/hour after initial bolus of 1.3 mg/kg/hour over 15 minutes), procain penicillin G (Procain Penicillin G®, 22.000 IU/kg IM once daily)[h], gentamicin (Gentacin®, 6.6 mg/kg IV once daily)[i], metronidazole (Metrobactin®,15 mg/kg orally every 8 hours)[i], flunixin-meglumine (Flunidol®, 0.5 mg/kg IV every 12 hours)[f] for 5 days and fluid therapy (Braun NaCl 0.9%®, saline 0.9% in combination with balanced electrolyte solution, Ursolyt 153S® 90 ml/kg/24 hours)[k,l]. Nasogastric intubation was performed every 6 hours during the first day, and duodenogastric reflux was not observed after surgery. Vital parameters were within normal ranges, borborygmi, defaecation and behaviour were normal. The horse was offered water and a handful hay and mash 24 hours postoperatively, but appetite was reduced. Gastroscopy was performed 4 days postoperatively. The stomach was filled with gastric content despite restricted feeding and fasting for 14 hours. Due to delayed emptying metoclopramide was administered (Metomotyl®, 0.25 mg/kg IM every 6 hours)[e] before gastroscopy was repeated the next day. The stomach was empty then and mild, equine glandular gastric disease (EGGD) was observed as multifocal, hyperemic lesions of the pylorus as well as mild inflammation of the duodenal mucosa. Duodenal biopsies revealed a neutrophilic inflammation with evidence of mucosal barrier disruption.

Medical therapy with betanechol (Myocholine Glenwood®, 0.05 mg/kg orally every 8 hours)[m] and sucralfate (Sucralfat®, 12 mg/kg every 12 hours)[n] was initiated. The horse started eating and showed normal appetite. Five days postoperatively, ventral oedema and mild purulent exudation at the incision site were apparent. Daily wound cleaning was performed.

Behaviour was normal and the remainder of the postoperative period was uneventful until discharge eight days postoperatively. The horse was fed with maintenance requirements of hay and mash at discharge, the owner was advised to start handwalking 5 minutes daily.

At re-examination four weeks later, wound healing was advanced. The owner reported of an uneventful recovery, without any signs of gastrointestinal disease. Gastroscopy still revealed mild EGGD of the pyloric region in addition to equine squamous gastric disease (ESGD), grade I/IV, gastric emptying was normal. Abdominocentesis was performed for monitoring and revealed turbid fluid with an elevated TNCC of 17.44×10^9/L (rr < 5.0×10^9/L) and elevated total protein of 40 g/L (rr < 20 g/L). Based on cytological findings, a modified transudate with a mixed cell population and small amounts of non-degenerated neutrophils was diagnosed without indication of septic inflammation. Serum AMY was < 10 IU/L, serum LIP was 13 IU/L, AMY in PF was 0 IU/L and LIP in PF

was 8 IU/L. Therapy with betanechol was tapered over 3 days and sucralfate was replaced by misoprostol (Cytotec®, 5 µg/kg every 12 hours)[o] for 3 more weeks.

Three weeks later, lesions of both the squamous and glandular mucosa showed almost complete healing at gastroscopy. Attempted abdominocentesis was unsuccessful, as peritoneal fluid could not be obtained. For evaluation of inflammatory processes, serum amyloid A (SAA) and fibrinogen were measured and within normal ranges. Misoprostol administration was continued for further gastric therapy for one week. The owner reported the horse doing well 3 months after discharge without any complaints.

Case 3

Case 3 was a 24-year-old Irish Tinker mare, presented with lethargy, sweating, signs of acute colic and severe neurologic deficits. The horse had a history of fever over 3 days up to 40 °C ten days before presentation and routine blood work performed by the referring veterinarian revealed markedly elevated liver enzymes. The day of referral, N-butylscopolamine and metamizole-natrium (Buscopan comp.®)[a] of unknown dosage was administered.

On arrival, the horse was somnolent and showed ataxia; vital parameters were within normal ranges. Oral mucous membranes were jaundiced, CRT was 2 seconds. CBC and SBC revealed haemoconcentration (PCV 0.47 L/L, rr 0.32–0.40 L/L), neutrophilic leucocytosis (WBC 11.25 G/L, rr 4.9–11.1 G/L; neutrophils 9.53 G/L, rr 2.5–6.9 G/L), lymphopenia (1.32 G/L, rr 1.5–5.1 G/L), markedly elevated ALKP (700 U/L, rr 10–326 U/L), elevated GGT (509 U/L, rr 0–87 U/L), hyperglobulinemia (64 g/L, rr 24–47 g/L), hyperglycemia (11.38 mmol/L, rr 3.55–8.32 mmol/L), hyperproteinemia (TP 93 g/L, rr 56–74 g/L), hyperbilirubinemia (59 g/L, rr 0–35 g/L) and severe hyperammonemia (276 µmol/L, rr 0–90 µmol/L). Bile acids were markedly increased (31.61 µmol/L, rr < 12 µmol/L, IDEXX reference laboratory). Transabdominal ultrasound examination showed mildly increased peritoneal fluid, fluid content within the colon ascendens and heterogenous echogenicity of the liver. Abdominocentesis revealed turbid PF with a normal TNCC (2.97×10^9/L, rr < 5.0×10^9/L) and TP (18 g/L, rr < 25 g/L), lactate was elevated (4.1 mmol/L, rr < 1.78 mmol/L). Diagnosis of hyperammonemic encephalopathy was made. AMY and LIP results were available the next day, serum enzymes were within normal ranges (AMY < 10 IU/L, LIP 9 IU/L), enzyme activities in PF were also low in comparison to reference intervals of serum (AMY 5 IU/L, LIP 19 IU/L). Treatment consisted of IV fluids (Braun NaCl 0.9 %®, saline 0.9 % in combination with balanced electrolyte solution, Ursolyt 153S® 90 ml/kg/24 hours)[k,l], penicillin (Penicillin-G-Natrium®, 22.000 IU/kg every 6 hours IV), gentamicine (Gentacin®, 6.6 mg/kg IV once daily)[l], metronidazole (Metrobactin®, 15 mg/kg every 8 hours PO)[i], flunixine-meglumine (Flunidol®, 0.25 mg/kg IV every 8 hours)[f] and lactulose sirup (Lactulose-ratiopharm®,1 ml/kg bwt every 8 hours PO) to decrease ammonium absorption. Neurologic signs progressed, the mare showed circling, aimless walking, head pressing and lack of orientation. Glaucoma of the right eye developed the following day, and euthanasia was elected.

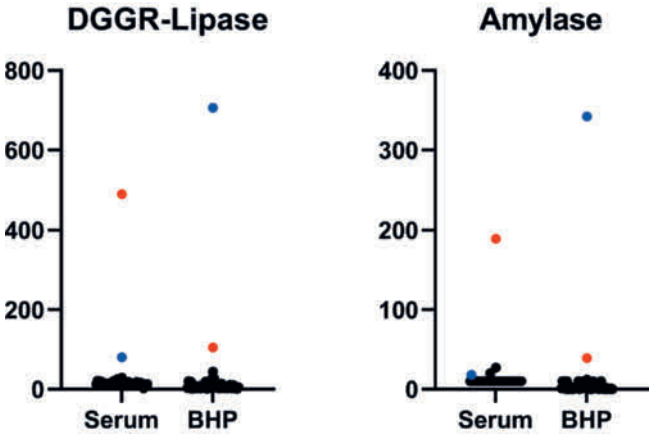

Fig. 1 Lipase has been elevated in case 1 (red) in serum and in case two (blue) in peritoneal fluid. Amylase as a less organ-specific enzyme has been higher in both cases, but still within normal ranges. *Lipase war bei Fall 1 im Serum erhöht (rot) und bei Fall 2 in der Peritonealflüssigkeit (blau). Amylase als weniger organspezifisches Enzym war bei beiden Fällen höher, aber noch im Referenzbereich.*

Tissue samples were taken immediately post-mortem from liver and pancreas for histopathologic examination. Aqueous humor from the right eye was also taken postmortem for measurement of ammonium which was markedly elevated (134 µmol/L, rr for serum ammonium < 90 µml/L, IDEXX reference laboratory). Histopathologic diagnosis was severe, chronic cholangiohepatitis with marked cirrhosis and chronic pancreatitis with mild fibrosis. Microbial culture performed from the liver tissue revealed *Clostridium butyricum* growth.

Discussion

In humans, a threefold lipase increase above the upper reference limit in conjunction with abdominal pain is required to make a diagnosis of acute pancreatitis (*Greenberg* et al. 2016). Diagnosis of chronic pancreatitis is more challenging and requires computed tomography and magnet resonance imaging (*Steer* et al. 1995, *Barry* 2018, *Singh* et al. 2019). Measurement of serum enzyme activities has a low sensitivity especially when large amounts of fibrotic tissue, which are unable to synthesize digestive enzymes, have replaced the normal pancreas (*Steer* et al. 1995). This may be an explanation for the third case in our study, in which pancreatic serum enzymes have been within normal ranges despite chronic inflammation and fibrosis found in histopathology. During the acute phase of the disease process, this case probably could have been detected by measurement of enzyme activities. An early diagnosis of acute pancreatitis in horses may help to guide therapy and thus prevent chronic disease stage.

In dogs and cats, acute pancreatitis is a common disease (*Watson* 2015, *Forman* et al. 2021) and diagnosis is based on ultrasonographic examination, serum lipase immunoreactivity (PLI) and cytology or histopathology of the pancreas (*Xenoulis* 2015). Measurement of canine pancreatic lipase immunoreactivity (cPLI) and lipase activities in PF also has diagnostic value in dogs with acute pancreatitis (*Chartier* et al. 2014).

In horses, measurement of enzyme activities currently seems to be the best diagnostic tool although there is a lack of information about the degree of increased pancreatic enzymes required to diagnose acute pancreatitis. In humans, AMY peaks rapidly after the onset of clinical signs, has a short half-life of 10–12 hours and returns to normal within three to five days (*Ismail* and *Bhayana* 2017). LIP rises within 3 to 6 hours after onset of signs, peaks within 24 hours and remains elevated for up to two weeks (*Lippi* et al. 2012). LIP is a large protein which cannot easily cross serosal or capillary barriers (*Chartier* et al. 2014), thus elevations of PF lipase may not be reflected in serum enzyme activity. Peritoneal fluid is an ultrafiltrate of plasma and constituents are dependent on different factors, which are Starling forces, vascular permeability, mesothelial lining integrity, and lympathic drainage (*Conrado* and *Beatty* 2021). Therefore besides cardiovascular and hydration state of the patient, fluid therapy may have a high impact on measured enzyme activities in S and PF. This is important for interpretation of results of the present study, as some horses already were treated with fluids before referral. Due to these physiologic properties and different time slots of AMY and LIP, repeated enzyme measurement in S and PF is required to monitor disease course and severity in horses. Whereas this is a preliminary study to investigate the occurrence of elevated pancreatic enzymes (AMY and LIP) in horses with gastrointestinal disorders in S and PF, measurements were performed only once. It is one limitation that measurement of enzyme activities has not been repeated following standardized time intervals.

Results show that pancreatic disease has a rare occurrence in horses, as enzyme activities were elevated in 2 out of 48 horses presenting with gastrointestinal or hepatic disease, in which S and PF could be obtained and in none of the control horses. Those 2 horses, presented with signs of acute colic, had moderately elevated LIP activity in serum (489 IU/L, rr < 250 IU/L, case 1) respectively peritoneal fluid (LIP 706 IU/L, case 2). The third case, with postmortem diagnosed chronic pancreatitis, revealed no elevated enzymes.

In recent research, LIP activity was measured in frozen plasma samples in 192 colic horses retrospectively and was shown to be elevated in 30.2 % of horses with gastrointestinal disorders (*Lanz* et al. 2022). Previously published reference values were used (*Desjardins* et al. 2017, *Johnson* et al. 2019) and an increased enzyme activity above 2 × upper reference limit occurred in 15.6 %, which was significantly associated with surgical treatment, strangulating disease and non-survival (*Lanz* et al. 2022). Patients presented due to colic were included, regardless of the underlying cause if blood samples had been submitted to the laboratory within 24 hours and if frozen left-over plasma samples were available (*Lanz* et al. 2022). In the present study, colic signs were not the criterion for inclusion in all cases, as some horses were presented with more subtle and unspecific signs of gastrointestinal or hepatic disease. Results suggest pancreatic damage to be a common secondary finding in gastrointestinal disease which occurs more frequently than primary pancreatitis does (*Yamout* et al. 2012, *Newman* 2015). These published reference intervals with plasma lipase activity < 21 IU/L (*Desjardins* et al. 2017, *Johnson* et al. 2019) and < 42 IU/L, respectively (*Lanz* et al. 2022) may deviate from reference intervals in our study probably due to different methods.

The present study has some major limitations. Enzyme measurements were performed in an external laboratory due to reasons of study implementation and results were available with time delay of 1–2 days. Suspicion of pancreatic disease based on elevated enzyme activities was not confirmed by histopathology and a definitive diagnosis has not been made. Both horses had mild disease courses and were discharged from hospital quickly, so further diagnostic procedures were not conducted. In man, approximately 80 % of acute pancreatitis cases have mild, self-limited disease and are discharged within several days (*Forsmark* et al. 2016). This mild course of acute disease may also occur in horses and contribute to equine pancreatitis as an under-diagnosed disorder.

Another limitation is that measurements of AMY and LIP have not been repeated for follow-up. Positive results were found in either serum or PF. Besides in 5 horses enzyme activities have been determined in S only, so elevated activities in PF may have been missed. The low incidence of elevated enzymes in the GI diseases group makes the chance of a positive case in the control group very low and thus the number of horses in the control group could have been higher. The low number of horses included is another limitation, further research with a larger population size is necessary.

Currently equine pancreatic disease is most commonly diagnosed postmortem. The obtained results in sixty-two horses revealed two horses that had elevated enzymes (S or PF) which might be indicative for secondary pancreatic disease. In future, routine inclusion of pancreatic enzymes into equine biochemistry profiles and PF examination as point-of-care examination could be helpful. It is a low-cost diagnostic procedure for acute pancreatic disease due to lack of other diagnostic options. The two patients recovered without any specific treatment, however, knowing that a horse might have a pancreatic disease additionally might change the therapeutic approach. Treatment of acute equine pancreatic disease should be performed corresponding to guidelines from humans and small animals.

Table 1 List of clinical cases with suspected pancreatic disease | Liste der klinischen Fälle mit dem Verdacht auf eine Pankreaserkrankung.

	sex	Age	breed	S AMY U/L	S LIP U/L	PF AMY U/L	PF LIP U/L	diagnosis	outcome
1	mare	20 y	Appaloosa	189	489	39	105	Gastric impaction	discharged
2	gelding	9 y	Haflinger	< 10	80	342	706	Duodenitis-proximal jejunitis, septic peritonitis	discharged
3	mare	24 y	Irish Tinker	< 10	9	5	19	Hyperammonemic encephalopathy, Chronic pancreatitis	euthanized

High sensitivity and specificity for pancreatic lipase in PF has been shown in dogs (*Chartier* et al. 2014), in horses further research is necessary.

In cases with elevated enzyme activities in S or PF, pancreatic biopsies should be performed in further studies. Improving diagnostic imaging modalities may also help in future for diagnosis of equine pancreatic diseases.

In conclusion, pancreatic disorders in horses may be underdiagnosed and may be missed due to mild courses which are not differentiated from gastrointestinal diseases. DGGR-Lipase determination from serum and peritoneal fluid is a least invasive, organ-specific, inexpensive and widely available tool which may contribute to diagnose acute pancreatic disorders in horses. Prospective evaluation of the utility of DGGR-Lipase for the prediction of pancreatic disease in a larger number of cases is required.

Conflict of interest statement

The study has been funded by IDEXX GmbH. Cora-C. Sommerey and Graham Stock are associates of IDEXX GmbH. The remaining authors have no conflicts of interest to declare.

Manufacturers´addresses

[a] Buscopan comp. ad us. vet. 500 mg/ml + 4 mg/ml Injektionslösung®: Boehringer Ingelheim Vetmedica GmbH, Binger Straße 173 , 55216 Ingelheim, Germany
[b] Torbugesic®Vet 10 mg/ml: Zoetis, Schellingstraße 1, 10785 Berlin, Germany
[c] Sonosite®: FUJIFILM Sonosite GmbH, Amelia-Mary-Earhart-Strasse 8, 60549 Frankfurt, Germany
[d] KARL STORZ SE & Co. KG, Dr.-Karl-Storz-Straße 34, 78532 Tuttlingen, Germany
[e] Metomotyl® 5 mg/ml: CP-Pharma Handelsgesellschaft mbH, Ostlandring 13, 31303 Burgdorf, Germany
[f] Flunidol RPS® 50 mg/ml: CP-Pharma Handelsgesellschaft mbH, Ostlandring 13, 31303 Burgdorf, Germany
[g] Lidocainhydrochlorid 2%®: bela- pharm GmbH & Co.Kg, Lohner Straße 19, 49377 Vechta, Germany
[h] Procain-Penicillin-G® 300,0 mg/ml: bela-pharm GmbH & Co.Kg, Lohner Straße 19, 49377 Vechta, Germany
[i] Gentacin® 85 mg/ml: bela- pharm GmbH & Co.Kg, Lohner Straße 19, 49377 Vechta, Germany
[i] Metrobactin® 500 mg Tabletten: Dechra Veterinary Products Deutschland GmbH, Hauptstr. 6–8, 88326 Aulendorf, Germany
[k] NaCl 0,9% B.Braun®: B.Braun Melsungen AG, Carl-Braun-Straße 1, 34212 Melsungen, Germany
[l] Ursolyt S153®: Serumwerk Bernburg Tiergesundheit GmbH, Hallesche Landstraße 105b, 06406 Bernburg, Germany
[m] Myocholine Glenwood 25 mg®: Glenwood GmbH, Arabellastraße 17, 81925 München, Germany
[n] Sucrabest® 1 g Tabletten: Combustin Pharmazeutische Präparate, Offinger Str. 3–7, 88525 Dürmentingen Germany
[o] Cytotec® 200 µg Tabletten: kohlpharma GmbH, Im Holzhau 8, 66663 Merzig, Germany
[p] Penicillin-G-Natrium® 180 mg/ml: bela- pharm GmbH & Co.Kg, Lohner Straße 19, 49377 Vechta, Germany
[q] Lactulose-ratiopharm® Sirup: ratiopharm GmbH, Graf-Arco-Straße 3, 89079 Ulm, Germany

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