Case Report

An accidental discovery of ductal adenocarcinoma of the pancreas in an adult horse with severe abdominal pain

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Abstract

A native pony, eight years old and male, was tentatively diagnosed with abdominal pain and treated immediately. Sequential hematological profiles revealed increased levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine, as well as reduced albumin. After the pony's death, which occurred eight days after treatment began, a necropsy was performed. The liver's external surface showed small foci, but no gross lesion was observed in the pancreas, which was enlarged. Haematoxylin and eosin (H&E) staining of the liver showed multifocal inflammation in the hepatic parenchyma, coinciding with bile duct hyperplasia. The pancreas H&E staining showed well-differentiated tubular glands with normal pancreatic islet morphology. The pancreatic immunohistochemical staining was positive for pan-cytokeratin in the cytoplasm, except in the pancreatic islet cells. It also confirmed negative chymotrypsin staining of the pancreatic tissue, indicating simple and branching ductal structures instead of acinar cells. The pancreas's histopathology was consistent with ductal adenocarcinoma, a condition previously unreported in adult equids. This result suggests that pancreatic tumors should be considered an uncommon differential diagnosis for abdominal pain in adult equids.

Keywords: Abdominal pain, Ductal adenocarcinoma, Horse, Pan-cytokeratin, Pancreas

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Introduction

Acute abdominal pain or colic is one of the most common problems requiring an emergency approach in equine practice (Curtis *et al*., 2019). Various gastrointestinal diseases can cause abdominal pain (Curtis *et al*., 2015); for example, spasmodic colic, large colon impaction, and displacement (Abutarbush *et al*., 2005). Several risk factors, including age, sex, breed, deworming program, and nutritional status, could contribute to colic in horses (Mehdi & Mohammad, 2006). Since there are similarities in clinical signs and case histories in horses presenting with abdominal pain, clinical diagnoses are still required to indicate the underlying cause of that pain.

Pancreatic adenocarcinoma, a debilitating disease causing poor body condition with abdominal pain, has previously been described in equids (Rendle *et al*., 2006; Spanton *et al*., 2009). Its significant clinical features encompass inappetence, weight loss, hyperbilirubinemia, and increased serum activity of hepatic enzyme markers (Rendle *et al*., 2006; Spanton *et al*., 2009). Pancreatic adenocarcinoma has predominantly been reported in aged equids, with no reports in adult equids. Moreover, acinar cell carcinomas of the pancreas have been differentiated histopathologically in aged equids (de Brot *et al*., 2014). However, the pancreatic cancer originating from ductal structures has yet to be described, particularly in adult equids. Therefore, this case report outlines the clinical and histopathological presentations of pancreatic ductal adenocarcinoma in an adult pony with severe abdominal pain.

Case description

An 8-year-old native pony (gelding, weighing 204 kg) was first presented to the equine practitioner with complaints including inappetence, long-term poor body condition, a lack of defecation, and persistent front limb pawing. The pony was housed in an individual stable within the barn and left in a paddock for approximately 2 hours daily. The pony was fed twice daily with commercial pellets totaling a kilogram and had free access to hay and tap water in its stable. Deworming with ivermectin oral formula and hoof trimming were performed at 60- and 30-day intervals, respectively.

Upon inspection, the pony showed poor body condition, scoring 3 out of 9 according to Kohnke's (Kohnke, 1992) modification method. Moderate depression, inappetence, occasional pawing, and rolling in the stable were also observed. The significant abnormal findings upon examination included increased heart rate (90 beats/min) and respiratory rate (32 breaths/min), 5% dehydration, and delayed capillary refilling time (CRT = 2 sec). Decreased gut motility with mild abdominal distension was also seen, concurrent with a resonant sound in the upper right and left of the abdomen. Rectal palpation revealed no impaction or distension of the large intestine. However, the rectal temperature (100.7 °F) was normal. The pony was diagnosed with abdominal pain tentatively resulting from ileus and gut obstruction.

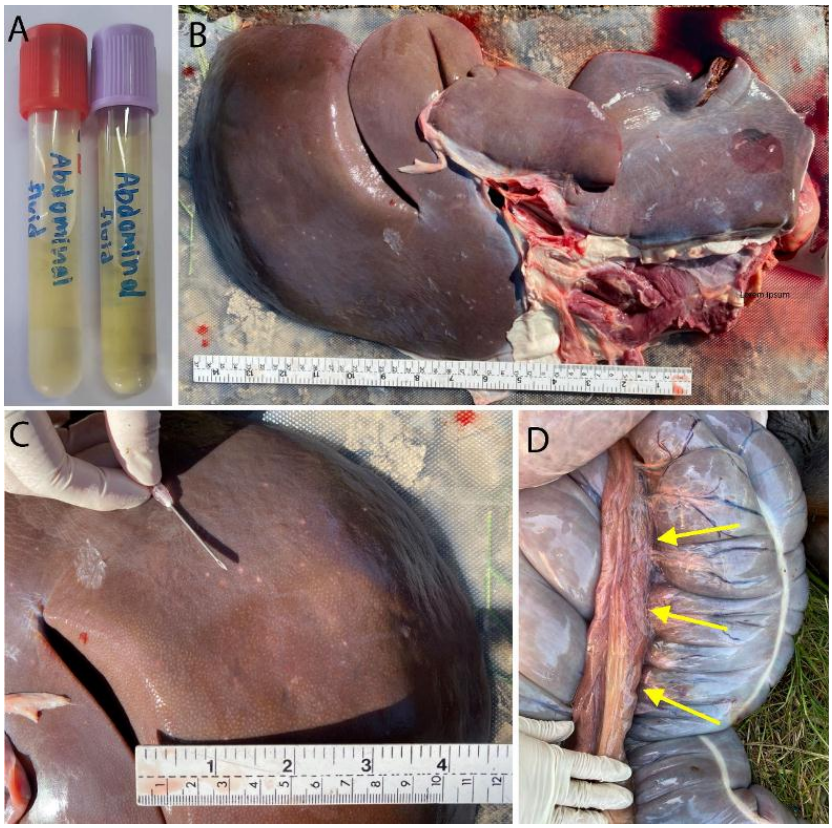

Figure 1 Gross lesion of specific abdominal organs after performing the necropsy. Yellowish fluid is collected from the abdominal cavity (A). The liver has a standard size without clear abnormal findings (B), except for the presence of multiple small white foci on its external surface (C). The enlargement of the mesenteric lymph nodes (yellow arrows) and the congested vessels of the exterior wall of the cecum are also observed (D).

Table 1 Hematological and serum biochemical parameters on Days 2, 6, 7, and 8 after treatment.

Blood parameters (normal ranges)	After treatment			
	Day 2	Day 6	Day 7	Day 8*
RBC (6.6 - 9.7 × 10^6/µL)	6.9	6.3	6.3	9.2
HCT (34% - 46%)	30.0	27	26	39
HGB (118 - 159 g/L)	105	96	99	142
MCV (43 - 55 fL)	42.9	42.1	42.4	42.5
MCH (15 - 20 pg)	15	15.1	15.6	15.4
MCHC (340 - 370 g/L)	350	354	368	363
RDW (16.3 - 19.3%)	17.6	17.6	17.9	17.7
WBC (5.2 - 10.1 × 10^3/µL)	9.8	10.2	10.9	12.8
NEU (2.7 - 6.6 × 10^3/µL)	7.84 (80%)	6.732 (66%)	7.63 (70%)	9.984 (78%)
LYM (1.2 - 4.9 × 10^3/µL)	1.764 (18%)	3.06 (30%)	3.052 (28%)	2.688 (21%)
MONO (0.0 - 0.6 × 10^3/µL)	0.196 (2%)	0.4 (4%)	0.109 (1%)	0.128 (1%)
EOS (0.0 - 1.2 × 10^3/µL)	0 (0%)	0 (0%)	0.109 (1%)	0 (0%)
PLT (94 - 232 × 10^3/µL)	242	358	320	288
AST (222 - 489 units/L)	>1500	1102	913	995
ALP (88 - 261 units/L)	965	676	580	504
Creatinine (70.74 – 132.63 µmol/L)	344.84	274.1	309.47	424.42
Total protein (52 - 78 g/L)	N/A	40	39	36
Albumin (29 - 36 g/L)	N/A	17	17	15
Globulin (23 - 38 g/L)	N/A	23	22	21
Total bilirubin (8.55 - 35.91 µmol/L)	N/A	27.36	25.82	28.90
Direct bilirubin (1.71 - 5.13 µmol/L)	N/A	12.83	15.05	10.26
Indirect bilirubin (5.13 - 34.2 µmol/L)	N/A	14.54	10.77	18.64
Bile acid (2 - 10 µmol/L)	N/A	N/A	9.59	10.64
Amylase (3 - 8 units/L)	N/A	N/A	N/A	9
DGGR lipase (7 - 16 units/L)	N/A	N/A	N/A	52

Results and Discussion

The pony showed no gastric reflux following nasogastric intubation. Ten liters of 0.9% normal saline solution were administered intravenously to address the dehydration, followed by intravenous flunixin meglumine at 0.5 mg/kg body weight. The pony was hand-walked after fluid therapy for 30 minutes every hour (for the following three hours) to stimulate gut motility. Small amounts of feces were excreted, and the gut sound was somewhat improved with hand walking. Intravenous flunixin meglumine was administered twice daily at 0.5 mg/kg body weight. Routine dental care revealed sharp edges of the cheek teeth, corresponding with irregular tooth wear. Inappetence persisted even though a floating procedure removed the sharp edges. A blood sample was collected on the second day of therapy and showed dramatic increases in aspartate aminotransferase (AST; > 1500 units/L), alkaline phosphatase (ALP; 965 units/L), and creatinine (344.84 µmol/L) levels (Table 1). These results indicated liver and renal malfunction concurrent with abdominal pain in the pony. The dose of flunixin meglumine was reduced to 0.25 mg/kg body weight twice daily, considering the suspected renal malfunction reflected by the increase in creatinine level (Divers, 2022). Normal hydration status and gut motility were observed as the pony could drink the water well. Ten liters of 0.9% normal saline solution were repeatedly done on days 3, 5, and 7. The pony was also given five capsules of a liver supplement (Heptacap, Mega Lifesciences, Bangkok, Thailand) three times daily to improve liver function. Sequential blood sampling on the sixth and seventh days of treatment showed reduced AST and ALP enzyme levels (Table 1). The pony was left in the paddock to be grass during the day. Even though acceptable gut sound was still detected with no abdominal distension, inappetence persisted, resulting in a lack of defecation during the seven days of treatment. In addition, the heart rate remained high, ranging from 70 to 95 bpm, throughout the period of the treatment.

The pony died early in the morning on the eighth day of treatment. A blood sample was withdrawn from the jugular vein immediately after the pony's death and revealed increased AST (995 units/L), ALP (504 units/L), and creatinine (424.42 µmol/L) levels, along with a high level of lipase activity (52 units/L; Table 1). A post-mortem examination revealed a clear yellowish fluid (Fig. 1A) trapped within the abdominal cavity. Cytological examination of the abdominal fluid revealed mixed inflammatory cells concurrent with clusters of polygonal epithelial cells without microorganisms. Non-septic peritonitis was suggested following the fluid investigation. Regarding the gross conformation of the liver, it had a standard size without clear abnormal findings (Fig. 1B), except for the presence of small white foci on its external surface (Figure 1C). Enlargement of the mesenteric lymph node and congested vessels on the caecal intestinal wall were also visualized (Fig. 1D). The pancreas was markedly enlarged and extended from the proximal duodenum to the liver's right lobe. Organ tissues, encompassing the pancreas, liver, and lung, were collected and fixed in 10% neutral buffered formalin for further histopathological analysis.

Histopathological evaluation of the pancreas tissue using hematoxylin and eosin (H&E) staining are shown in Figs. 2A and B. The histopathological presentation showed cuboidal to columnar shape neoplastic cells characterized by oval vesicular nucleus, 2-4 prominent nucleoli, pleomorphic, pale eosinophilic cytoplasm rearranging as well-differentiated tubular pattern (black arrows). Atypical mitosis of the cells and normal pancreatic islet morphology were revealed, and pancreatic adenocarcinoma was suspected. The liver tissue

revealed multifocal inflammation in the hepatic parenchyma (Figs. 2C-F). Immunohistochemical (IHC) staining using paraffin-embedded sections of the pancreas was then performed to differentiate between ductal and acinar adenocarcinoma. The pancreatic tissue revealed diffuse strongly positive pan-cytokeratin (multi-cytokeratin clone AE1/AE3, Leica Biosystems, Newcastle, UK), a valuable marker of epithelial malignancies (Barak *et al*., 2004) in the form of dark brown color in the cytoplasm of the cells, except for the pancreatic islet cells (Figs. 3A and B). Furthermore, the pancreatic tissue appeared to be negative via IHC staining for chymotrypsin (Chymotrypsin C/CTRC Antibody, Novus Biologicals, Colorado, USA) (Figs. 3C and D), an enzyme secreted by the pancreatic acinar cells (Sigel & Klimstra, 2013), suggesting that pancreatic ductal adenocarcinoma was present in this pony.

Horses with an age of over 15 years are considered aging (Williams, 2000), in which tumors and other malignancies have been diagnosed as an underlying cause of abdominal pain (Rendle *et al*., 2006; Spanton *et al*., 2009). In the present case, those abnormalities were not primarily differentiated due to the young age of the pony. Colic accompanying ileus and gut obstruction were initially diagnosed based on the clinical signs of inappetence, suspicious abdominal pain (indicated by pawing), a marked increase in heart rate, and decreased gut motility (Cook & Hassel, 2014). Although the pony demonstrated more alertness, concurrent with improving gut motility, after physical therapy and body fluid restoration during the first 24 hours and on days 3 and 6 of treatment, inappetence coinciding with a considerably higher heart rate than normal persisted. These symptoms could reflect pain (Reid *et al*., 2017).

Since the pony had satisfactory intestinal sounds and could drink water, the possible source of pain related to intestinal malfunction was believed to be lessened. According to previous studies, abdominal pain can originate from intestinal component abnormalities and liver disease (Klohnen, 2012). Accordingly, AST and ALP levels were analyzed on the second treatment day to differentiate the potential source of pain from liver disease. A substantial increase in the liver biomarkers, indicating hepatocellular injury, was observed in this pony, similar to previous reports (Satué *et al*., 2022). Although a reduction in AST and ALP levels was noted following the administration of the oral liver supplement, the values remained higher than normal throughout the treatment period, indicating that the hepatic injury persisted. The presence of conjugated hyperbilirubinemia, which indicates hepatocellular disease, cholestasis, and pancreatic or biliary malignancies (Tripathi & Jialal, 2022), supported the presence of hepatocellular injury in this pony. Moreover, an increase in 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methyl resorufin) ester (DGGR) lipase activity was detected on the eighth treatment day (52 units/L; Table 1), relating to pancreatic inflammation and, in part, to hepatic disorder (Lim *et al*., 2022). Thus, the hepatic and pancreatic diseases leading to inappetence and abdominal pain were further differentiated. A liver biopsy was planned on the eighth day, simultaneous with abdominal ultrasonography, to investigate the occurrence of tumors or cancer. However, the pony died in the early morning of the eighth day, after which the necropsy was performed.

As reported previously, abundant evidence of intraabdominal lesions has been detected in aging equids with pancreatic tumors, encompassing turbid serosanguinous abdominal fluid (Spanton *et al*., 2009), enlargement of the liver, spleen and mesenteric lymph node and multinodular masses on the pancreas and proximal duodenum (Rendle *et al*., 2006). Notably, no distinct masses were evident within the pancreas at the post-mortem examination, but the pancreas was markedly enlarged, similar to the report described previously (Carrick *et al*., 1992). Despite the histopathological investigation suggesting pancreatic adenocarcinoma, IHC staining of the pancreas was further performed to differentiate the origin of the pancreatic tumor.

IHC staining of cytokeratin, an intermediate filament protein subfamily, is widely used to differentiate human epithelial malignancies because it reflects ongoing cell activity (Narayan *et al*., 2022). It was also applied to identify epithelial derivation in a duodenal mass in a horse with metastatic pancreatic adenocarcinoma (Rendle *et al*., 2006). In contrast, trypsin and chymotrypsin are helpful, highly sensitive markers of acinar cell carcinoma (Sigel & Klimstra, 2013). Based on the pan-cytokeratin positivity (Figs 3A and B) and chymotrypsin negativity (Figs. 3C and D), as well as the negative chromogranin staining (data not shown) in the pancreatic tissue, in this case, ductal adenocarcinoma of the pancreas was suggested.

In summary, the clinical presentation of inappetence, poor body condition, and abdominal pain in this pony, along with the increases in liver and pancreatic biomarker levels, was consistent with previous reports indicating pancreatic adenocarcinoma in aging equids. Ductal adenocarcinoma of the pancreas, which has never been reported in equids, was differentiated in this adult pony. Therefore, pancreatic tumors should be considered an uncommon differential diagnosis for abdominal pain in adult equids.

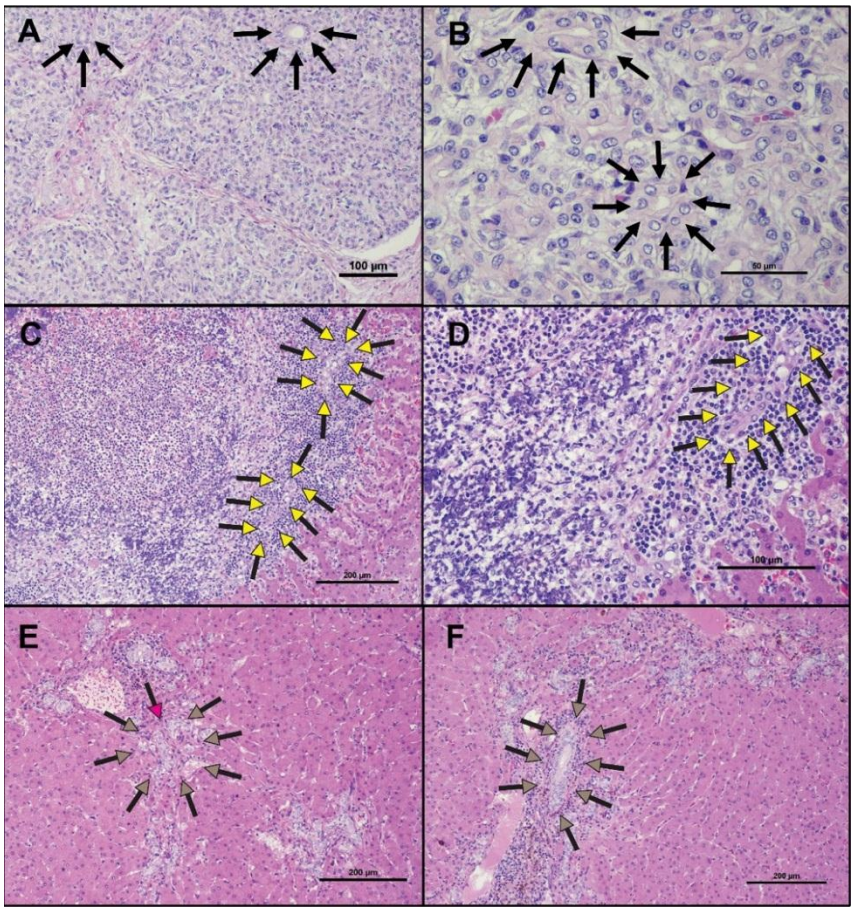

Figure 2 Histopathological examination of the pancreas and liver using hematoxylin and eosin staining. A and B present pancreatic tissue captured at 10x and 40x magnification, respectively. The mass shows well-differentiated tubular glands (black arrows) with normal pancreatic islet morphology. C-F presents the staining images of liver tissue that reveal diffuse multifocal inflammation in the hepatic parenchyma. Ductal structures (yellow-headed arrows) are also observed in the inflammatory areas at 10x (C) and 20x (D) magnification. E and F show images of bile duct hyperplasia at 10x magnification with atypical ductal architecture (pink-headed arrows) present in several areas of hepatic tissue, such as the portal, central, and peri-lobular structures of the liver.

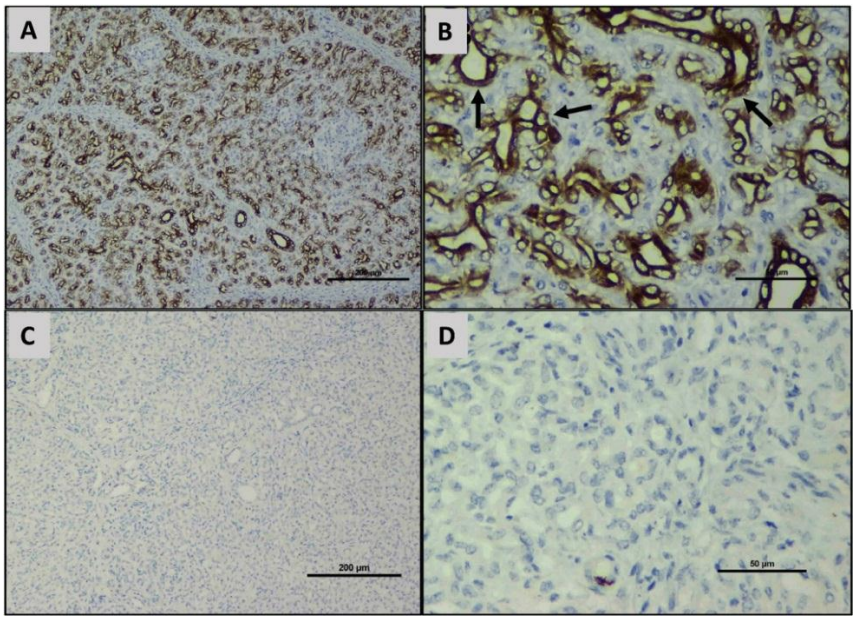

Figure 3 Immunohistochemical staining of pancreas tissues to differentiate between acinar and ductal adenocarcinoma. The images in the left and right panels are captured at 10x and 40x magnification, respectively. A-B demonstrates pan-cytokeratin staining of pancreatic tissue sections, represented by a dark brown color (black arrows). The diffuse strongly positive pan-cytokeratin stain is marked in the cytoplasm of the pancreatic tissue, except in pancreatic islet cells. C and D depict negative chymotrypsin staining of the pancreatic tissue sections, indicating not acinar but ductal adenocarcinoma of the pancreas.

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