Pferdeheilkunde – Equine Medicine 34 (2018) 6 (November/December) 531-537 DOI 10.21836/PEM20180604

Liver disease associated with leptospirosis in a mare

Julieta Born[1], Carsten Rohde[1], Peter Wohlsein[2], and Carlos E. Medina-Torres[3]

[1] Pferdeklinik am Kottenforst.
[2] Department of Pathology, University of Veterinary Medicine Hannover.
[3] Equine Specialist Hospital, School of Veterinary Science, The University of Queensland.

Summary: A nine-year-old Trakehner mare was presented as an emergency due to colic, and with a history of intermittent fever and poor performance. A questionable titre for Leptospira interrogans serovars Bratislava (L. Bratislava) and Pomona and a negative titre for Babesia caballi were documented. Clinical examination revealed tachycardia, pyrexia, delayed capillary refilling time, reduced gastrointestinal borborygmi, copious reflux upon nasogastric intubation, and no abnormalities upon per-rectum abdominal palpation. Trans-abdominal ultrasonography revealed congestion of the biliary tree with thickening of the ductal walls, and distention and amotility of the duodenum. Severe neutrophilic leucocytosis, liver enzyme elevation, increased bile acids, hypertriglyceridaemia, total hyperbillirubinaemia, hyperproteinaemia and hyperglycaemia were present. A positive titre for L. Bratislava and Australis, and a negative titre for L. Pomona were identified. Intravenous fluid therapy was initiated and the volume of nasogastric reflux gradually declined overnight; no further colic signs were observed. A liver biopsy was obtained and histological findings included severe hepatic fibrosis with bile duct proliferation and cholestasis, multifocal mild neutrophilic hepatitis, and hepatosis with glycogen storage. Aerobic and anaerobic bacterial cultures were negative. Repeated blood analysis after one week demonstrated an increase in antibody titres against L. Bratislava and further increase in gamma-glutamyltranferase (GGT) and alkaline phosphatase. A presumptive diagnosis of liver disease associated with leptospirosis was made and therapy with procaine penicillin initiated. The mare remained clinically stable and was discharged with instructions to continue therapy for an additional 2 weeks. Six weeks after discharge the mare had remained clinically stable and all but one liver parameter had normalised. The antibody titre against L. Bratislava had declined to undetectable levels. Six months after discharge a complete recovery with return to previous performance levels was reported. Though extensively described in various other animal species, as well as in humans, the association between liver disease and leptospirosis in horses is poorly characterised. Despite the lack of a strong association between liver damage with Leptospira spp. infection in horses, leptospirosis due to acute infection or potential recrudescence of leptospiraemia should be considered a differential diagnosis in cases with clinical signs and laboratory abnormalities compatible with hepatopathy, as well as in horses with chronic, recurrent, mild fever. This case report describes the history, clinical presentation, diagnostic test results and response to treatment that support a diagnosis of Leptospira-induced hepatopathy in a mare.

Keywords: Leptospira, L. bratislava, hepatic disease, hepatopathy, horse, equine

Citation: Born J., Rohde C., Wohlsein P., Medina-Torres C. E. (2018) Liver disease associated with leptospirosis in a mare. Pferdeheilkunde 34, 531-537; DOI 10.21836/PEM20180604

Correspondence: Julieta Born, Pferdeklinik am Kottenforst, Beckers Kreuz 25, 53343 Wachtberg, Germany; mvzjulietapr@gmail.com. Carlos Medina, Equine Specialist Hospital, Veterinary Medical Centre, School of Veterinary Science, Faculty of Science, Gatton Campus, The University of Queensland, Gatton, QLD 4343, Australia; c.medina@uq.edu.au

Introduction

Leptospirosis is a global zoonotic disease first reported in horses in 1947 (*Swan* et al. 1981). It is caused by the spirochete bacterium Leptospira, and has rarely been reported in equines (*Verma* et al. 2013). Due to this low incidence, the knowledge about leptospirosis in horses is less than in many other domestic species (*Donahue* and *Williams* 2000). The organism can be transmitted through infected urine, body fluids, and contaminated soil and water (*Smith* 2015), and in many instances, infection is clinically inapparent. Clinical features of the disease recognised in horses include fever, jaundice, anorexia, lethargy and poor performance (*Smith* 2015, *Twigg* and *Hughes* 1971, *Verma* et al. 2013, *Donahue* and *Williams* 2000, *Swan* et al. 1981), and it has been commonly associated with abortion, stillbirth, placentitis, renal disease, equine recurrent uveitis and immune-mediated keratitis (*Verma* et al. 2013, *Donahue* and *Williams* 2000, *Swan* et al. 1981). Hepatitis and nephritis have rarely been reported in adult horses (*Twigg* and *Hughes* 1971), which is in contrast with findings in other species including humans, dogs and pigs, where histopathologic changes in the liver and kidneys are characteristic (i.e. scattered hepatic necrosis, neutrophilic periportal hepatitis, and non-suppurative interstitial nephritis, with degenerative inflammatory dissociation (*Sykes* et al. 2011, *Levett* 2001, *Tochetto* et al. 2012, *De Brito* et al. 1966). Interestingly, though seldom associated with signs of hepatopathy in adult horses (*Konrád* and *Vošta* 1968), pregnancy losses due to leptospirosis are characterised by microscopic lesions in the liver, kidneys, lungs and heart of the aborted foetus (*Verma* et al. 2013, *Donahue* and *Williams* 2000, *Szeredi* and *Haake* 2006). *Twigg* and *Hughes* (1971) observed that 65.2% of animals showing icterus, fever, poor performance and evidence of liver damage carried agglutinins to Leptospira spp., suggesting a role for this bacterium in the aetiopathogenesis of hepatitis and nephritis, as it indeed does in various other domestic animal species (*Twigg* and *Hughes* 1971).

In addition, the diagnosis of Leptospira spp. infection can proof challenging, with many reported difficulties in reaching definitive confirmation of the diagnosis (*Swan* et al. 1981). This is due, at least in part, to remaining uncertainty about disease incidence and serovar prevalence (*Verma* et al. 2013), the lack of specific clinical signs, difficulty and lack of consensus in result interpretation of single-time-point serological tests, and the initiation of treatment prior to sampling for diagnostic testing (*Swan* et al. 1981). Despite these difficulties, serological tests remain the most frequently used diagnostic aid to support a presumptive diagnosis of leptospirosis. Of these, the microscopic agglutination test (MAT) is the most

widely used (*Smith* 2015, *Verma* et al. 2013, *Donahue* and *Williams* 2000, *Ahmad* et al. 2005, Swan et al. 1981), and a four-fold or greater increase in antibody titres is considered as evidence of recent infection in the horse. In the presence of clinical signs, the patient should be treated and isolated. Therapy includes supportive treatment and the use of antibiotics (e.g. penicillin, amoxicillin, doxycycline) to control and attempt to eliminate infection (*Smith* 2015, *Donahue* and *Williams* 2000). Furthermore, though culture and identification of the infecting Leptospira sp. isolate remain the gold standard (*Verma* et al. 2013), it is well accepted that the diagnosis of leptospirosis by culture is impractical; i.e. it is labour-intensive, taking up to 6 months to grow the bacterium and requiring specialised laboratory procedures to identify an isolate (*Donahue* and *Williams* 2000, *Ahmad* et al. 2005, *Carter* and *Davis* 2007). At present, the association between liver disease and leptospirosis in horses remains weak at best, and though occasionally suspected, the disease is poorly characterised in equine medicine (*Sykes* et al. 2011, *Levett* 2001). The case presented here describes a mare with liver disease associated with rising antibody titres demonstrating seroconversion against Leptospira interrogans serovar Bratislava (hereafter L. Bratislava) that responded well to therapy.

Case description

A nine-year-old Trakehner mare was presented as an emergency due to colic, and with a history of intermittent fever and poor performance of six months duration. Four days prior to presentation, complete blood count (CBC) and serum chemistry performed by the referring veterinarian identified a normal total leucocyte count (7.8×10^9/L [reference interval (RI): $5–14\times10^9$/L]), with a mild lymphopaenia (0.85×10^9/L [RI: $1–7\times10^9$/L]), elevated liver enzymes , and increased total bilirubin (Table 1, Day -4). Titres for L. Bratislava and Pomona of 1:400 and <1:100, respectively (RI: <1:400 negative, 1:400 questionable, >1:400 positive), and a negative titre for *Babesia caballi* (<1:80; RI: <1:80 negative, 1:80 low, 1:160 medium, >1:160 high) were documented. Treatment with vitamin B, glucose and oxytetracycline was initiated. The day prior to presentation oral doxycycline was administered; a moderate to severe colic was subsequently observed, where nasogastric intubation yielded four litres of gastric reflux. The mare was treated with flunixin meglumine, metamizole (dipyrone), dexamethasone, buthorphanol and detomidine, and was referred to the hospital.

On presentation, the mare was quiet, alert and responsive, rectal temperature was 38.6°C, heart rate was 48 beats per minute, respiratory rate was 20 breaths per minute, oral mucous membranes were congested, capillary refilling time was 3 seconds, and reduced gastrointestinal borborygmi were identified. An additional 15 L of gastric reflux were obtained upon nasogastric intubation, and per-rectum abdominal palpation revealed no abnormalities. Trans-abdominal ultrasonography revealed distention of the biliary tree, thickening of the portal triads (Fig. 1), and distention and amotility of the duodenum (Fig. 2). The initial differential diagnoses for a mare with a history of fever, colic, gastric reflux, elevated liver enzymes and the clinical findings at presentation included acute or chronic liver failure associated with bacterial (e.g. Leptospira sp.), viral (e.g. Hepacivirus or Pegivirus [Theiler's disease]) or parasitic (e.g. flukes or larval migration) infectious cholangiohepatitis, cholelithiasis or toxic insults (e.g. pyrrolizidine alkaloids), as well as duodenitis-proximal jejunitis, strangulating small intestinal lesions, and large colon displacement and/or volvulus. The latter were ruled out based on per-rectal abdominal palpation.

A CBC and serum chemistry revealed a severe leucocytosis (19.31×10^9/L [RI: $5–14\times10^9$/L]), with neutrophilia (17.6×10^9/L [RI: $2–8\times10^9$/L]), confirmed the severe liver enzyme elevation and total hyperbilirubinaemia, and demonstrated an increase in serum bile acids (SBA) (Table 1: Day 0), hypertriglyceridaemia (1.57 mmol/L [RI: 0,2–1,13 mmol/L]), hyperproteinaemia (92 g/L [RI: 57–75g/L], and hyperglycaemia (13.9 mmol/L [RI: 4,9–6.2 mmol/L]); urea, creatinine and electrolyte concentrations were within reference range. Faecal floatation and sedimentation egg counts were negative. Anti-Leptospira antibody titres on MAT identified a 1:800 titre for serovar Bratislava, <1:100 for serovar Pomona, and

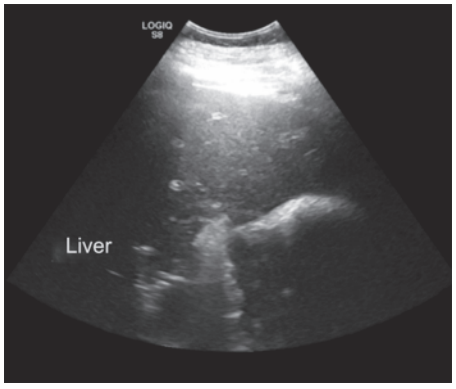

Fig. 1 Ultrasonography of the liver obtained with a 3.5-MHz probe at a frequency of 3.0 MHz, over the right eighth intercostal space and a displayed depth of 22 cm. Left of the image is dorsal. Distention of the biliary tree and mild thickening and hyperechogenicity of the walls of the bile ducts, compatible with calcification is apparent. *Sonogramm der Leber im achten Interkostalraum auf Höhe der Scapula, durchgeführt mit einem 3,5-MHz Schallkopf mit einer Frequenz von 3,0MHz und einer Eindringtiefe von 22 cm. Links ist dorsal. Sichtbar ist eine Distension/Erweiterung des Gallengangsystems, eine geringgradige Verdickung und Hyperechogenität der Gallengangswände, entsprechend einer Kalcifizierung.*

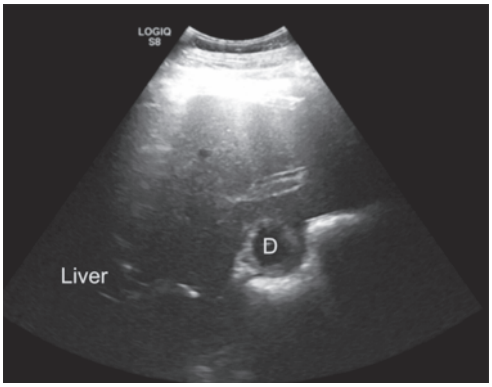

Fig. 2 Ultrasonography of the liver obtained with a 3.5-MHz probe at a frequency of 3.0 MHz, over the right eleventh intercostal space and a displayed depth of 22cm showing the liver as described in Fig. 1, and a mildly dilated duodenum (D). Left of the image is dorsal. *Sonogramm der Leber im elften Intercostalraum durchgeführt mit einem 3,5- MHz Schallkopf mit einer Frequenz von 3,0 MHz und einer Eindringtiefe von 22 cm. Sichtbar ist die Leber und ein geringgradig erweitertes Duodenum. (D). Links ist dorsal.*

1:800 for serovar Australis. An ELISA test for Fasciola hepatica was negative and no Lepstospira spp. DNA was identified in the sample (real time-PCR was negative).

Intravenous fluid therapy (Ursolyt 153S®) at 1.5 maintenance rate (80 ml/kg per day) was initiated. Thirty litres of nasogastric reflux were recovered overnight and no further colic signs were identified. Partial reestablishment of duodenal motility was confirmed ultrasonographically, nasogastric reflux gradually decreased over the following 48 h, and on day three water and small amounts of feed were reintroduced. Oral omeprazole (2 mg/kg q 24 h; Gastrogard®) was added to the therapy, and nutritional supplementation with a multivitamin-mineral formulation (Viverosan Komplex B, Derbymed®) and a commercial product for horses suffering from liver insufficiency (Legaphyton, Vetoquinol®) were given with feed. Following a coagulation profile, which yielded normal partial thromboplastin time, prothrombin time, thrombin clotting time and fibrinogen results, a liver biopsy was collected and submitted for histopathological and bacteriological examination.

For histopathology, biopsy samples were fixed in 10% neutral formalin and processed routinely into paraffin wax. Tissue sections of 4 µm thickness were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS)-reaction, Turnbull's blue, Azan- and Fouchet-stains, and Warthin-Starry silver impregnation. Histologically, the biopsies displayed a regular lobular architecture of the liver parenchyma. There were multifocal mild infiltrations of neutrophilic granulocytes. Numerous partially coalescing foci of hepatocytes were present with a moderately swollen faintly eosinophilic fine granular cytoplasm surrounded by prominent cell margins and a small nucleus (Fig. 3). The PAS-reaction resulted in intense cytoplasmic staining indicating marked glycogen storage. Mild accumulations of brownish pigment were present in hepatocytes that stained positive for bile pigment. In addition, multifocal small accumulations of bile were found in biliary canaliculi. Iron accumulation was not detected. Broad septa of connective tissue rich in collagen fibres were present, often associated with multifocal, moderate bile duct proliferation (Fig. 4). Under polarised light, collagen fibres showed intense birefringence. Silver impregnation did not reveal any spiral-shaped bacteria. The histological diagnoses included severe hepatic fibrosis with bile duct proliferation and cholestasis, multifocal mild neutrophilic hepatitis and hepatosis with glycogen storage. Aerobic and anaerobic bacterial cultures were negative.

Repeated blood analysis after one week demonstrated a further increase in GGT and ALP, and a decrease in AST, GLDH), SBA and total billirubin (Table 1, Day 7). A gradual increase in MAT antibody titres against L. Bratislava was confirmed, from 1:400 before referral, to 1:800 on the day of presentation to the hospital, and up to 1:1,600 on day 12 of hospitalisation.

A presumptive diagnosis of liver disease associated with leptospirosis was made based on the raising antibody titres against L. Bratislava, and therapy with procaine penicillin (22,000 IU/kg IM q12 h) was initiated. The mare remained clinically stable and was discharged after four additional days in hospital with instructions to continue therapy with penicillin for two more

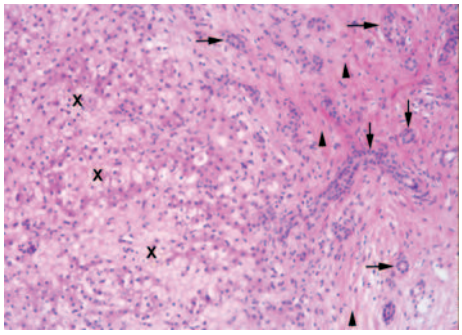

Fig. 3 Liver biopsy displaying multiple foci of swollen hepatocytes (X) with faintly eosinophilic cytoplasm and distinct cell margins suggestive of glycogen storage. Adjacent severe fibrosis (arrowheads) with multifocal bile duct proliferation (arrows) is present. HE, magnification 100x.
In der Leberbiopsie sind multiple Herde mit geschwollenen Hepatozyten (X) erkennbar, die ein schwach eosinophiles Zytoplasma und deutliche Zellgrenzen aufweisen und wahrscheinlich Glykogen gespeichert haben. Angrenzend ist eine hochgradige Fibrose (Pfeilspitzen) mit zahlreichen proliferierten Gallengängen (Pfeile) vorhanden. HE, Vergrößerung 100x.

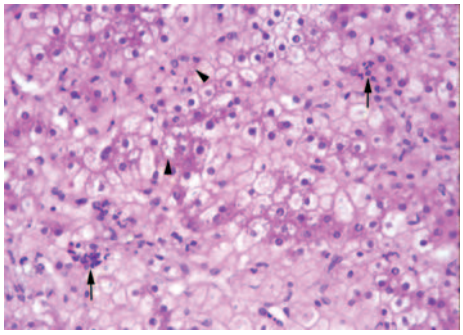

Fig. 4 Higher magnification showing small foci of neutrophilic infiltration (arrows) and mild accumulation of fine granular brownish pigments in hepatic cytoplasms (arrowheads). HE, magnification 200x.
Die höhere Vergrößerung zeigt kleine, herdförmige Infiltrationen mit neutrophilen Granulozyten (Pfeile) und eine geringgradige Akkumulation eines feingranulären braunen Pigmentes im Zytoplasma von Leberzellen (Pfeilspitzen). HE, Vergrößerung 200x.

Table 1 Serum concentrations of liver enzymes, serum bile acids (SBA) and total bilirubin.

Parameter	Day -4	Day 0	Day 7	6 weeks	RI
AST	644	742	503	304	138-409 IU/L
GGT	394	850	8,666	121	8-22 IU/L
ALP	N/A	1,474	1,474	172	86-285 IU/L
GLDH	167	244	244	12.4	0-12 IU/L
Bilirubin	173	193	51	22.2	8.5-39.3 µmol/L
Bile acids	N/A	82.1	14.3	4.9	1-11 µmol/L

RI: reference interval; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; ALP: alkaline phosphatase; GLDH: glutamate dehydrogenase; N/A: not assessed.

weeks, monitor hepatic markers in serum and repeat MAT antibody titres for Leptospira spp. four weeks after discharge.

Case Outcome

Six weeks after discharge the mare remained clinically stable, with a normal demeanour and behaviour, a good appetite and no recorded fever. Except for an elevation in GGT (121 IU/L [RI: 8–22 IU/L]), serum chemistry performed by the referring veterinarian demonstrated normalisation of all other measured liver parameters (Table 1, 6 weeks), including triglycerides (0.46 mmol/L [RI: 0.2–1.13 mmol/L]), and total protein (67 g/L [RI: 57–75 g/L]). The antibody titre against L. Bratislava on MAT had declined to undetectable levels (<1:100 [RI: negative <1:100]); a titre for serovar Australis was not measured. On further follow-up six months later, the owner reported a complete recovery of the mare, with return to previous performance levels. Serum chemistry and complete blood count results were completely normal at the time, except for a chronically elevated GGT (113 U/L [RI: 8–22 IU/L]), which remained almost unchanged since six weeks after discharge.

Discussion

The diagnosis of hepatic disease in the horse can be difficult as clinical signs are often non-specific and only become apparent when greater than 70–80% of the liver parenchyma has been damaged (*Gehlen* et al. 2010). Furthermore, the list of differential diagnoses that need to be considered before attempting a diagnosis of liver disease is extensive and includes multiple toxic, infectious, metabolic, degenerative, obstructive and idiopathic causes (*Divers* 2012).

In the case reported here, the main clinical signs identified included intermittent fever and poor performance of several months duration, and a sudden onset of colic; fever is most consistent with bacterial cholangiohepatitis or cholelithiasis (*Smith* 2015), whilst abdominal pain has been associated with hepatomegaly or with obstruction of the biliary tree due to choleliths (*Gehlen* et al. 2010, *Smith* 2015). Delayed gastric emptying as well as intestinal hypomotility has also been associated with liver disease, particularly if the bile ducts are affected (*Gehlen* et al. 2010).

Severe elevation of all measured liver parameters was identified in this case. Gamma-glutamyltranferase is the most sensitive indicator of liver disease in the horse (*Smith* 2015) and was highly elevated in the case presented here. Values above 250 IU/L are typically seen, when severe liver damage has occurred. Together with elevations in SBA, a severe elevation in GGT is associated with intrahepatic or post-hepatic cholesthasis (*Gehlen* et al. 2010). The severe elevation in ALP, which characterises chronic liver disease in the horse, further supported the diagnosis of sustained severe hepatic disease in the mare.

The clinical signs, laboratory test results and ultrasound findings further corroborated the presence of liver disease in the mare. Serologic testing for various pathogens associated with acute and chronic hepatic disease in horses was performed, and a liver biopsy was collected in an attempt to identify the aetiologic cause of liver damage and define a specific therapy. Histology of the liver biopsy revealed severe hepatic fibrosis with bile duct proliferation and cholestasis, indicative of extensive chronic damage as suggested by the severe elevation in all measured liver parameters. A multifocal mild neutrophilic hepatitis was also identified; hepatic infiltration of neutrophils is an acute response to recent or ongoing liver injury, hepatic stress or unknown systemic inflammatory signals. In humans, after the initial phase of bacteraemia, Leptospira spp. invade several organs including the liver. Recruitment of neutrophils into inflamed tissues is a complex and coordinated sequence of events that are mandatory for pathogen elimination in the affected tissue (*Loic* et al. 2016, *Ruonan* et al. 2014). This could explain the mild neutrophilic hepatitis in this mare. Fascioliasis was ruled out based on parasitologic and serologic testing for Fasciola hepatica, together with the history and management of the mare. Pyrrolizidine alkaloid (PA) toxicity is characterised by weight loss, moderate icterus and abnormal behaviour, all of which were absent in this mare. The triad of fibrosis, bile duct proliferation and megalocytosis, which characterises PA toxicity histologically (*Smith* 2015), was not identified in this mare. Furthermore, horses that develop clinical signs of liver failure following PA intoxication usually die within 5 to 10 days. The continuous intake of other toxic substances like phytotoxins, mycotoxins and chemicals may also result in clinical signs of chronic active hepatitis in the horse, which is characterised by chronic progressive hepatocyte necrosis and fibrosis, in addition to signs of colic, fever, icterus and sometimes inflammation of the coronary band (*Gehlen* et al. 2010); the ingestion of such toxicants cannot be ruled out with certainty, however, history and management of the mare make such an intoxication highly unlikely.

The differential diagnoses for hepatic disease in horses also include viral infections such as equine infectious anaemia (EIA), equid herpesvirus-1 (EHV-1), equine viral arteritis (EVA), Theiler's disease, and equine hepacivirus (initially known as non-primate hepacivirus (npHV)) infection (*Cavalleri* and *Feige* 2016, *Ramsay* et al. 2015, *Brehm* et al. 2017). Theiler's disease had long been associated with the administration of equine blood products 4 to 10 weeks prior to clinical disease, but an identical clinical presentation has been observed in horses without a history of blood product administration (*Orsini* and *Divers* 2014). It has recently been associated with a viral agent of the Pegivirus genus in the Flaviviridae family, capable of causing acute necrotising hepatitis and hepatic failure (*Cavalleri* and *Feige* 2016, *Orsini* and *Divers* 2014, *Chandriani* et al. 2013). Furthermore, a recent metagenomics analysis has documented the detection of viral DNA material of a new virus (named Kirkovirus by the study authors) in the liver of a horse with severe fatal idiopathic hepatopathy with histopathological findings compatible with Theiler s disease (*Li* et al. 2015). In addition, Theiler's disease has also been more recently associated with a parvovirus; namely the equine parvovirus hepatitis (EqPV-H) virus (*Divers* et al. 2018). Clinical signs and clinicopathological findings are those of acute hepatic failure (increased liver enzymes and bile acids – as in the case presented here – hepatic encephalopathy and prolonged PT and PTT) (*Cavalleri* and *Feige* 2016, *Orsini* and *Divers* 2014). Despite the recent association of these viruses with the occurrence of Theiler's disease, diagnostic tests for these infections are not readily available in diagnostic centres and were therefore not performed in this case. However, the occurrence of Theiler's disease was deemed unlikely as the histopathological findings

of centro-lobular to mid-zonal hepatocellular necrosis with haemorrhage, which characterises Theiler's disease, were not identified in the mare (*Orsini* and *Divers* 2014, *McAuliffe* and *Knottenbelt* 2014, *Sprayberry* and *Robinson* 2015).

The MAT serology titre of 1:400 for L. Bratislava before referral is reported as questionable by the laboratory. However, after 12 days in hospital a titre of 1:1600 was identified. While the presence of Leptospira antibodies in the horse has been suggested to be more meaningful compared to other species (*Konrád* and *Vošta* 1968), a four-fold or greater rise in antibody titres between acute and convalescent sera in the presence of clinical signs is considered diagnostic of recent active Leptospira spp. infection (*Chirathaworn* et al. 2014, *Sykes* et al. 2011). *Twigg* and *Hughes* (1971) observed that 65.2% of sick horses showing icterus, fever, poor performance and evidence of liver damage carried agglutinins to Leptospira spp. They went on to suggest that Leptospira spp. could play some causative part in the pathogenesis of hepatitis and nephritis in horses (*Twigg* and *Hughes* 1971). Since then, Leptospira interrogans infection in the horse has been established as an aetiologic cause of uveitis, placentitis, abortion, stillbirth, renal disease and haemolysis (*Smith* 2015, *Verma* et al. 2013), and clinical signs include fever, anorexia, poor performance, listlessness and icterus (*Smith* 2015, *Verma* et al. 2013, *Donahue* and *Williams* 2000). Microscopic lesions in aborted foetuses include suppurative and non-suppurative nephritis, hepatocellular dissociation, leucocytic infiltration and neutrophil granulocytic infiltration in the liver parenchyma and in the portal triads, giant cell hepatopathy, pulmonary haemorrhages, pneumonia, and myocarditis (*Verma* et al. 2013, *Donahue* and *Williams* 2000, *Szeredi* and *Haake* 2006).

In a study on 292 horses, antibody titres against Leptospira spp. were assessed using an agglutination-lysis reaction that identified positive results in 73% of horses with liver cirrhosis; of these, 42.3% had a titre of 1:800 or higher (*Konrád* and *Vošta* 1968). In cases with acute hepatopathy (enzootic hepatitis, icterus gravis, hepatodystrophia) or other acute liver lesions, 64.2% and 80% respectively, had titres between 1:100–1:400. Overall, 49% had a titre of 1:800 or higher (*Konrád* and *Vošta* 1968). Interestingly, in the case presented here, an increase between the acute and convalescent antibody titre from 1:400 to 1:1600 for L. Bratislava was identified in association with moderate to severe histopathologic lesions indicative of liver damage in a biopsy specimen. Together, these findings are highly suggestive of hepatopathy as a result of leptospirosis in the mare.

Unfortunately, confirming a diagnosis of leptospirosis in the horse can be challenging, despite multiple diagnostic modalities being described. Dark ground microscopy and serology represent the basis for diagnosis in most instances. In the case presented here, a MAT was performed. The MAT is considered reliable, has a quick turn-around time, and is currently the most widely used and accepted test for the serologic diagnosis of Leptospira spp. infection (*Smith* 2015, *Verma* et al. 2013, *Donahue* and *Williams* 2000, *Ahmad* et al. 2005, *Sykes* et al. 2011, *Bernard* 1993). It is readily available and inexpensive, and its use is supported by a large body of data (*Sykes* et al. 2011), with detection of serum antibodies reported as early as one week after the onset of clinical signs (*Bourhy* et al. 2011, *Levett* 2001).

Culture followed by identification of the infecting leptospiral isolate remains the gold standard for the diagnosis of Leptospira spp. infection (*Verma* et al. 2013). However, it is impractical as it is labour-intensive, has a slow turn-around time (6 months to grow), and requires a specialised laboratory and experienced personnel to identify the isolate (*Donahue* and *Williams* 2000, *Ahmad* et al. 2005, *Carter* and *Davis* 2007). While spirochete bacteria are found in the bloodstream in the first few days after exposure, albeit at very low levels, PCR of blood samples could rapidly confirm the diagnosis in the early phase of disease and before antibody titres are detectable, which would provide a more convenient diagnostic alternative (*Verma* et al. 2013). However, due to the very low leptospiraemia that characterises infection, thus requiring very sensitive molecular diagnostic methods (*Bourhy* et al. 2011), no satisfactory protocol has yet been developed for routine application in diagnostic laboratories (*Donahue* and *Williams* 2000), and most PCR assays are unable to identify the infecting serovar (*Ahmad* et al. 2005). Further-more, blood sampling prior to or within two days of initiating antibiotic therapy has been recommended, as antibiotics are said to quickly eliminate leptospiraemia (*Bourhy* et al. 2011). However, as PCR detects DNA from both viable and nonviable organisms, in patients with leptospiraemia, DNA detection should be plausible even after multiple doses of antimicrobials (*Sykes* et al. 2011). As the mare presented here received tetracyclines for four days prior to referral, this may explain why PCR was negative. Furthermore, at least in dogs where leptospiraemia is high during the initial 10 days of infection, blood is the sample of choice only during the first week of illness (*Sykes* et al. 2011). With the six- month history of recurrent fever and poor performance, it is also likely that the acute phase of infection in this mare, and therefore leptospiraemia, had occurred months prior, which could also explain why Leptospira spp. DNA was not detected.

In humans, a diagnosis of leptospirosis is made in every suspicious case with a positive PCR, a positive culture, seroconversion with MAT from two samples within 10 days, or any combination of these (*Moral* 2014). In domestic animals, the diagnosis of leptospirosis is generally based on the MAT, with a 4-fold increase in titre accepted as evidence of recent infection. In horses, elevated serum antibody titres are observed after the leptospiraemic phase, and rising titres after two to three weeks are considered diagnostic. In the presence of clinical signs compatible with leptospirosis, the patient should be isolated and treated (*Smith* 2015). The mare presented here showed a four-fold increase in antibody titres against L. Bratislava together with signs consisted with liver disease. Leptospira interrogans serovar Bratislava is considered the host-adapted serovar of the horse (*Smith* 2015, *Donahue* and *Williams* 2000, *Ellis* et al. 1983); albeit some existing controversy, it has been associated with disease and is considered pathogenic to the horse, despite being host-adapted (*Smith* 2015, *Twigg* and *Hughes* 1971).

Treatment for leptospirosis in equids has been mostly extrapolated from other species, with little or no species-specific information available for horses (*Bernard* 1993, *Verma* et al. 2013). Therapy of liver failure is usually supportive, targeted at preventing further liver damage and to promote liver regeneration (*Gehlen* et al. 2010). In suspected cases of leptospirosis in horses, the use of ampicillin, amoxicillin, procaine penicillin, oxytetracycline or doxycycline for seven days or

more has been advocated (*Smith* 2015, *Donahue* and *Williams* 2000, *Sykes* et al. 2011). Furthermore, the use of penicillin or streptomycin has been suggested as the treatment of choice against leptospirosis in horses, with the caveat that the latter poses potential toxic side effects in this species (*Verma* et al. 2013, *O'Neill* 2012, *Prescott* 1991).

Following resolution of colic and gastric reflux, and after reaching a working diagnosis of hepatopathy likely due to leptospirosis, the mare was treated with procaine penicillin for two weeks. The mare responded well to treatment, and a reduction in MAT titres for L. Bratislava was confirmed after six weeks. This rapid decline in antibody titre occurred faster than previously documented with reduction in antibodies against L. Bratislava occurring over two to six months in clinical cases with confirmed liver damage, poor performance, and low grade fever (*Twigg* and *Hughes* 1971). However, none of these horses received treatment against Leptospira spp. After six weeks, all liver enzymes had returned to normal except for GGT, which remained elevated at 121 IU/L, but demonstrated a marked decrease from greater than 8,000 IU/L prior to discharge (Table 1). In large animal species, an elevation in serum GGT is considered one of the most reliable indicators of liver damage and biliary obstruction, and once present, the levels may remain elevated for several weeks (*Smith* 2015). The moderate elevation that remained was therefore expected. Other blood parameters were within reference ranges and the mare was clinically healthy.

Whether a different acute disease process predisposes horses to recrudescence and clinical manifestations of latent leptospirosis, or acute leptospirosis is responsible for secondary disease occurrence, and this secondary disease is the one diagnosed, remains to be determined (*Konrád* and *Vošta* 1968). This mare presented for acute colic and copious gastric reflux, which was compatible with a diagnosis of small intestinal disease. Whether this acute event of small intestinal disease was secondary to chronic leptospirosis which could explain the protracted history of fevers and poor performance, or an isolated event that precipitated recrudescence of leptospirosis leading to acute hepatopathy cannot be established. Therefore, the major limitation of this case reported is the lack of confirmation of a leptospirosis diagnosis based on identification of the presence of Leptospiras or their DNA in a sample from the mare. Furthermore, positive antibody titres can be detected in healthy horses, making interpretation of serology testing difficult. Thus, the diagnosis of leptospirosis in this mare remains presumptive. However, the demonstration of a rising antibody titre and confirmation of a four-fold increase in the titre against serovar Bratislava during hospitalisation strongly suggests active infection with this serovar. With the protracted history of previous fever and malaise, the acute hepatic disease process upon presentation to the hospital was attributed to recrudescence of infection rather than primary leptospiral colonisation of the patient. This recrudescence was likely secondary to a primary ailment that could have remained undiagnosed and may have resolved with the instituted therapeutic regime (e.g. anterior enteritis with clostridial involvement, which could have been at least in part addressed by the penicillin treatment that was administered to the mare).

The possibility of a misdiagnosis due to lack of diagnostic testing for recently reported viral aetiologies of hepatitis in the horse (*Cavalleri* and *Feige* 2016, *Ramsay* et al. 2015, *Brehm* et al. 2017) must be acknowledged. However, the clinical presentation of the case reported here, together with the clinicopathological and histopathological findings, all highly suggestive of primary liver disease, together with the identification of a rapidly rising titre against L. Bratislava, the response to therapy and the subsequent decline in antibody titres six weeks after treatment, are highly supportive of a working diagnosis of leptospirosis as the cause of hepatopathy in this mare.

In summary, the history, clinical presentation, diagnostic test results and response to treatment, support a diagnosis of Leptospira-induced hepatopathy in this mare. This case report therefore supports the somewhat forgotten recommendation from *Twigg* and *Hughes* (1971) to include leptospirosis as a differential diagnosis in horses with chronic, recurrent mild fever (*Twigg* and *Hughes* 1971). Furthermore, despite the lack of a strong association between liver damage with Leptospira spp. infection in horses, leptospirosis due to acute infection or potential recrudescence of leptospiraemia in previously infected animals should be considered a differential diagnosis in cases with clinical signs and laboratory abnormalities compatible with hepatopathy. Confirmation of the diagnosis should involve exclusion of other infectious and non-infectious causes of hepatic disease, determination of acute and convalescent antibody titres, and detection of leptospiral DNA in blood or tissue samples.

References

Ahmad S. N., Shah S., Ahmad F. M. H. (2005) Laboratory diagnosis of leptospirosis. Postgrad. Med. 51, 195-200

Alventosa M. C., Plana Campos L., Larrey Ruíz L., Acedo Mayordomo R., Sanchís Artero L., Peño Muñoz L., Núñez Martínez P. C., Castillo López G. A., Latorre Sánchez M., Urquijo Ponce J. J., Dlago Madrud M., García-Argüelles J. C. (2017) Gastrointestinal bleeding and acute hepatic failure by leptospirosis: an entity that should not be forgotten. Rev. Gastroenterol. Peru 37, 96-99

Bourhy P., Bremont S., Picardeau M. (2011) Comparison of Real-Time PCR assays for detection of pathogenic Leptospira spp. in blood and identification of varations in target sequences. J. Clinic. Microbiol. 49, 2154-2160; DOI 10.1128/JCM.02452-10

Brehm W., Gehlen H., Ohnesorge B., Wehrend A. (2017) Handbuch Pferdepraxis. Enke Verlag. Stuttgart

*Carter G. R., Davis E. (2007) Microbial Diseases: G through L. A Concise Guide to t*he Microbial and Parasitic Diseases of Horses. International Veterinary Information Service.

Cavalleri J. M. V., Feige K. (2016) Proceedings of the European Veterinary Conference Voorjaarsdagen 2016

Chandriani S., Skewes-Cox P., Zhong W., Ganem D. E., Divers T. J., van Blaricum A. J., Tennant B. C., Kistler A. L. (2013) Identification of a previously undescribed divergent virus from the Flaviridae family in an outbreak of equine serum hepatitis. PNAS 110, 1407-1415; DOI 10.1073/pnas.1219217110

Chirathaworn C., Inwattana R., Suwancharoen D. (2014) Interpretation of microscopic agglutination test for leptospirosis diagnosis and seroprevalence. Asian Pac. J. Trop. Biomed.4, 162-164; DOI 10.12980/APJTB.4.2014C580

De Brito T., Freymüller E., Hoshino S., Penna D. O. (1966) Pathology of the kidney and liver in the experimental leptospirosis of the guinea-pig. Virchows Arch. Path. Anat. 341, 64-78; DOI 10.1007/BF00959245

Divers T. (2012) Diagnosis and treatment of liver disease. BEVA Congress

Divers T. J., Tennant B. C., Kumar A., McDonough S., Cullen J., Bhuva N., Jain K., Chauhan L. S., Scheel T. K. H., Lipkin W. I., Laverak M., Trivedi S., Srinivasa S., Beard L., Rice C. M., Burbelo P. D., Renschaw R. W., Dubovi E., Kapoor A. (2018) New Parvovirus associated with serum hepatitis in horses after inoculation of com-

mon biological product. Emerging infectious diseases. 24, 303-310; DOI 10.3201/eid2402.171031

Donahue J., Williams N. M. (2000) Emergent causes of placentitis and abortion. Vet. Clin. North Am. Equine Pract. 16, 443-456; DOI 10.1016/S0749-0739(17)30088-3

Ellis W. A., O Brien J. J, Cassells J. A., Montgomery J. (1983) Leptospiral infection in horses in Northern Ireland: Serological and microbiological findings. Equine Vet. J. 15, 317-320; DOI 10.1111/j.2042-3306.1983.tb01809.x

Gehlen H., May A., Venner M. (2010) Lebererkrankungen beim Pferd. Pferdeheilkunde 26, 668-679; DOI 10.21836/PEM 20100501

Konrád J., Vošta J. (1968) Das Verfolgen der Antikörpertiter gegen Leptospiren bei Hepatopathie und anderen inneren Krankheiten der Pferde. Deutsch. Tierärztl. Wschr. 75, 347-352

Levett P. N. (2001) Leptospirosis. Clin. Microbiol. Rev. 14, 296-326; DOI 10.1128/CMR.14.2.296-326.2001

Li L., Giannitti F., Low J., Keyes C., Ullmann L. S., Deng X., Aleman M., Pesavento P.A., Pusteria N., Delwart E. (2015) Exploring the virome of diseases horses. J. Gen. Virol. 96, 2721-2733; DOI 10.1099/vir.0.000199

Loic R., Giry C., Vandroux D., Kuli B., Randrianjohany A., Pequin A., Renou F., Jaffar- Bandjee M., Gasque P. (2016) Major neutrophilia observed in acute phase of human leptospirose is not associated with increased expression of granulocyte cell activation markers. PLoS One. 11. DOI 10.1371/journal.pone.0165716

Mcauliffe S., Knottenbelt D. (2014) Color Atlas of Diseases and Disorders of the Horse. Saunders Elsevier

Moral M. (2014) Enfermedades infecciosas leptospirosis: Diagnóstico de Leptospirosis. Guía para el equipo de salud 9. Ministerio de Salud de la Nación, República Argentina

O'Neill H. (2012) An overview of aminoglycoside usage in the horse. Companion Animal banner 17, 4-7; DOI 10.1111/j.2044-3862.2012.00248.x

Orsini J. A, Divers T. J. (2014) Equine Emergencies: Treatment and Procedures. Elsevier Saunders. Missouri

Prescott J. (1991) Treatment of leptospirosis. Cornell Vet. 81, 7

Ramsay J. D., Evanoff R., Wilkinson Jr. T. E., Divers T. J., Knowles D. P., Mealey R. H. (2015) Experimental transmission of equine hepacivirus in horses as a model for hepatitis C virus. Hepatology. 61, 1533-1546; DOI 10.1002/hep.27689

Xu R., Huang H., Zhang Z., Wang F. S. (2014) The role of neutrophils in the development of liver diseases. Cell. Mol. Immunol. 11, 224-231; DOI 10.1038/cmi.2014.2

Smith B. (2015) Large animal internal medicine. 5th Ed. Mosby Elsevier. St. Louis, Missouri

Sprayberry K. A., Robinson N. E. (2015) Robinson s Current Therapy in Equine Medicine. Elsevier Saunders. Missouri

Swan R. A., Williams E. S, Taylor E. G. (1981) Clinical and serological observations on horses with suspected leptospirosis. Australian Vet. J. 57, 528-529; DOI 10.1111/j.1751-0813.1981.tb 05798.x

Sykes J. E., Hartmann K., Lunn K. F., Moore G. E., Stoddard R. A., Goldstein R. E. (2011) 2010 ACVIM Small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, an prevention. J. Vet. Intern. Med. 25, 1-13; DOI 10.1111/j.1939-1676.2010.0654.x

Szeredi L., Haake D. (2006) Immunohistochemical identification and pathologic findings in natural cases of equine abortion caused by leptospiral infection. Vet. Pathol 43, 755-761; DOI 10.1354/vp.43-5-755

Tochetto C., Flores M. M., Kommers G. D., Barros C. S. L., Fighera R. A. (2012) Pathological aspects of leptospirosis in dogs: 53 cases (1965-2011). Pesq. Vet. Bras. 31, 430-443

Twigg G. I., Hughes D. M. (1971) Ocurrence of Leptospirosis in Thoroughbred Horses. Equine Vet. J. 3, 52-55. DOI 10.1111/j.2042-3306.1971.tb04440.x

Verma A., Stevenson B., Adler B. (2013) Leptospirosis in horses. Vet. Microbiol. 167, 61-66; DOI 10.1016/j.vetmic.2013.04.012