CPD article

Equine liver disease in the field. Part 1: approach

Liver disease in adult horses is commonly identified during investigation of non-specific clinical signs such as general malaise, lethargy or weight loss. In some cases, disease may be advanced and irreversible by the time a diagnosis is reached. Serum biochemistry and tests of liver function form an important part of diagnosing liver disease but provide limited information regarding aetiology, severity and prognosis. Liver biopsy is recommended in the majority of cases to confirm the presence of disease, to guide therapeutics and to provide information regarding prognosis.

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Liver disease is commonly encountered in adult horses and reaching an early diagnosis is key to ensuring a successful outcome. However, the prevalence of subclinical disease combined with a lack of pathognomic clinical signs make recognition challenging, and disease is often advanced by the time a diagnosis is made. In the first of two articles we provide an overview of the approach to liver disease in the field. Part 2 will focus specifically on causes and management of liver disease in adult horses.

Liver structure and function

The equine liver comprises 1.5% of body weight (Dyce et al, 2002) and contains up to 10% of the total blood volume at any one time. It has multiple functions including metabolism of proteins, carbohydrates and lipids; production of coagulation factors, plasma proteins and acute phase proteins; synthesis of vitamin C and storage of other vitamins; elimination of by-products (including ammonia and bilirubin); detoxification; phagocytosis; and drug metabolism by P450 enzymes. A close connection with the gastrointestinal tract via the hepatic portal vein means that the liver is regularly exposed to enteric-derived insults.

Clinical signs of liver disease

Liver disease can be classified as focal or generalised, and acute or chronic. Clinical signs of disease can be subtle and varied, and are often absent in the early stages of disease. With a reserve of approximately 80%, liver damage must be advanced before clinical signs occur and the organ begins to fail. Onset of clinical signs is therefore often abrupt, even with chronic disease processes. There may be a progression from liver disease to liver failure, particularly with chronic conditions, and it is important to make this distinction. If hepatocyte loss is gradual and regeneration occurs at a similar rate, failure is less likely to follow. The prognosis for horses with mild hepatic disease is good, if an early diagnosis is made. Once damage is sufficient to cause failure, prognosis becomes markedly worse. Common non-specific clinical signs of liver disease include poor performance, lethargy, colic, anorexia, icterus and weight loss. Haemorrhage, hepatic encephalopathy, bilateral laryngeal paralysis, photosensitisation and diarrhoea may also be seen, and may be severe enough to warrant referral.

Diagnosis

Clinicopathological findings

Serum biochemistry is an invaluable tool for the diagnosis of liver disease. Its widespread use in the investigation of non-specific clinical signs such as lethargy and general malaise often identifies increases in liver enzymes, suggestive of disease. However, this provides little information regarding severity, prognosis and cause. Testing liver function provides further information regarding the severity of disease and may help to guide prognosis.

- **Gamma glutamyl transferase (GGT)** is produced by the biliary epithelium and is increased in cholestasis or biliary injury. It may also be released into plasma with hepatocellular injury (Noonan, 1981). This enzyme has a relatively long half-life, remaining elevated for several weeks and may continue to rise despite clinical improvement, particularly in acute disease. Lung, kidney, pancreas and mammary gland also produce small amounts of GGT — however, these quantities are negligible, so GGT is considered liver specific. Mild to moderate increases in GGT (up to 140 IU/L) are commonly seen in Thoroughbred racehorses in training, possibly due to ongoing hepatic injury in response to training load or induction of GGT following repeated glycogen depletion and repletion during intensive training (Mack et al, 2014). This has been positively correlated

with cumulative days in training and, when ≥100 IU/L, has been associated with poor performance (Snow and Harris, 1998). Increased GGT has also been documented in other clinically normal performance horses (Divers, 2015). Increased GGT activity is a normal finding in foals, as well as in donkeys and mules, in which it can be increased up to 3 times the normal reference range for horses. Increases may also be seen with gastrointestinal disease, especially right dorsal displacement of the large colon and proximal small intestinal disease causing biliary outflow obstruction (Gardner et al, 2005). As a result, mild to moderate increases in GGT alone are commonly identified on serum biochemistry in apparently healthy horses. If liver function markers are normal, GGT can be re-analysed in 2–4 weeks. Should activity continue to increase, or should other indicators of liver disease be identified, further diagnostics could be considered. Sampling of 2–3 herd mates could also be considered in these situations, to rule out possible toxic or infectious insults.

- **Sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GLDH)** are both liver-specific and are increased with hepatocellular injury. They have shorter half-lives than GGT (SDH 12 hours, GLDH 14 hours) and return to baseline in 3–5 days following an acute insult. Both are useful for monitoring progression and resolution of disease. Note that GLDH tends to have a greater magnitude of increase and is more stable than SDH (Meyer and Raquel, 2013); SDH is stable for up to 24 hours if refrigerated. In one study, SDH was found to be elevated in 46% of 802 Thoroughbred racehorses, although the significance of this is unknown (Ramsey et al, 2019).
- **Aspartate aminotransferase (AST)** is commonly increased in hepatic disease and reflects hepatocellular injury. It is non-specific to the liver, as it is also produced in skeletal and cardiac muscle. This enzyme can be increased by haemolysis, as it is present within erythrocytes, and by lipaemia. Raised levels of AST should be interpreted with other liver-specific enzymes and creatine kinase, to help rule out muscle as a source of increased enzyme activity (Divers and Barton, 2018).
- **Alkaline phosphatase (ALP)** reflects biliary injury but is non-specific to the liver, also being produced in bone, intestine and macrophages. Additionally, ALP may be increased in pregnancy. Increased activity should be examined in conjunction with liver-specific enzymes; contribution from other sources should be considered (Pearson, 1999).
- **Lactate dehydrogenase (LDH)** is non-specific unless the iso-enzyme (LDH-5) is measured. It is rarely used in a clinical setting (Divers and Barton, 2018).
- **Alanine aminotransferase (ALT)** is commonly used as a marker of hepatocellular injury in dogs and cats but has a much lower activity in large animals and ALT therefore is not analysed in equids. Instead, GLDH and SDH are used (Divers and Barton, 2018).

Markers of liver function include:

- **Serum bile acids:** these are an excellent screen of liver function. Approximately 90% of circulating bile acids are normally removed by the enterohepatic circulation; serum levels may therefore be increased in liver disease. Increases up to 20 µmol/L can also be seen with prolonged fasting (Hoffman et al, 1987) and with intestinal disorders, but levels greater than 25–30 µmol/L are specific for liver disease or dysfunction, and indicate a poor prognosis in chronic cases (Dunkel et al, 2015). Importantly, this is not the case in acute diseases such as cholangiohepatitis. In these cases, bile acids will often decrease in association with clinical improvement, therefore serial measurements are useful to assess progression or response to treatment.
- **Bilirubin:** this pigment is not a sensitive indicator of liver function and can be normal in many cases, especially with chronic disease (McGorum et al, 1999). Elevations can be caused by increased production or by impaired uptake, conjugation or excretion by the liver. Haemolysis causes increased production of bilirubin, while fasting leads to impaired uptake by the liver, increasing the circulating levels. Measuring the conjugated (direct) versus unconjugated (indirect) fractions may provide more information as to the source of the increase. Greater than 25% of total bilirubin as the conjugated fraction suggests hepatocellular or hepatobiliary disease (Peek and Divers, 2000), but spill-over of conjugated bilirubin into the circulation can occur if large amounts are being processed by the liver. Increased levels should be interpreted carefully in conjunction with history and other clinicopathological results.
- **Ammonia levels** are an alternative test of liver function and are increased in almost all cases of hepatic encephalopathy. There does not appear to be a correlation between the magnitude of increase and the severity of liver disease (McGorum et al, 1999). Although hyperammonaemia is a sensitive indicator of liver disease in the horse, it is not specific; ammonia can also be increased in non-hepatic diseases such as gastro-intestinal hyperammonaemia (Dunkel et al, 2011). Note that samples need to be processed immediately or levels will become falsely elevated. Ammonia measurement is not offered by many referral laboratories for this reason, making it less useful as a first-line test in the field. However, samples can often be taken to a human hospital to be run. Samples should be collected anaerobically in EDTA tubes, and if immediate analysis is not possible, plasma should be spun, separated and frozen.
- **Bromsulphthalein (BSP) dye clearance** is a dynamic test of liver function. Clearance is prolonged with reduced function (Divers and Barton, 2018). This test is not commercially available at present.

Other clinicopathological changes include:

- **Hypoalbuminaemia:** albumin is synthesised by the liver and serum concentration was found to be lower in non-survivors (Dunkel et al, 2015). It has a long half-life and hypoalbuminemia is uncommon in liver disease, particularly in mild cases.
- **Hyperglobulinaemia:** beta globulins may be increased due to loss of Kupffer cells (liver-specific macrophages) that play a role in immune function and respond to the multitude of enteric derived challenges to which the liver is exposed. Increases in globulin concentrations indicate a substantial insult, and globulins have been shown to be higher in non-survivors (Dunkel et al, 2015).

- **Increased iron:** hepcidin is a hormone synthesised by the liver that controls dietary iron absorption by ferroportin (Oliveira Filho et al, 2010). Disruption of hepcidin upregulates ferroportin and increases iron absorption. Poor mechanisms for excreting iron exist; these are limited to loss of skin cells or haemorrhage.
- **Reduced blood urea nitrogen (BUN):** failure to convert ammonia to urea results in reduced BUN in some cases. This is not typically seen in mild or subclinical disease (Divers and Barton, 2018).

Imaging

Ultrasonography is a helpful tool if an abnormality is identified, but a normal ultrasound does not rule out even significant hepatic disease. Ultrasonographic examination can be performed in conjunction with percutaneous biopsy (see below). A low-frequency (2.5–5 MHz) transducer is required; higher-frequency tendon and rectal probes do not provide adequate depth in adult horses. Clipping of the hair coat is not usually necessary if adequate wetting of the coat with alcohol is performed, as long as the horse does not have an excessive hair coat. The liver should be imaged from both sides of the abdomen and is typically found between intercostal spaces (ICS) 5–9 on the left and ICS 8–15 on the right in clinically normal horses (Johns and Miles 2016). It is not always visible on the left side. This cranial location within the abdomen means that a proportion of the liver is obscured by the lung fields, making it impossible to visualise in its entirety (*Figure 1*). Liver dimensions may be reduced in older horses, with atrophy of the right lobe typically seen. This is thought to be a result of chronic compression by the right dorsal colon and caecal base (Dyce et al, 2002). It can be useful to compare the liver to the adjacent spleen on the left side: healthy liver tissue is hypoechoic relative to the spleen, with a more obvious vascular pattern (*Figure 2*). Bile ducts are not visible in normal liver.

Ultrasonography may reveal dilatation of bile ducts; biliary stones; hepatic enlargement; rounded edges; abscesses; masses; and changes in echogenicity (increases in echogenicity may be seen with fibrosis). Incidental diffuse hyperechoic granulomas may be identified ('starry sky' pattern, *Figure 3*). These are likely associated with previous parasitic infection and can be differentiated from choleliths by their extrabiliary location (Carlson et al, 2011). Hydatid cysts may be visualised as circular anechoic structures and are generally considered an incidental finding.

Biopsy and histopathology

Percutaneous liver biopsy is indicated if liver disease is suspected but history, clinical signs and laboratory findings are not indicative of a specific disease. It is arguably the most valuable prognostic technique in the diagnosis of liver disease, and may help to guide treatment. It has been shown to have a better specificity for long-term survival than serum bile acids (Dunkel et al, 2015). Abnormal coagulation profiles are common in horses with liver disease, but do not appear to be associated with complications when performing biopsy (Johns and Sweeney, 2008). Platelet counts in these horses are generally normal (Divers, 2015). Complications associated with biopsy include haemorrhage, pneumothorax, pleuritis, peritonitis, colic and inadvertent sampling of another organ, but these are generally rare. Biopsy can theoretically be performed blind in the 14th intercostal space at the intersection of a line drawn from tuber coxae to point of elbow. However, this poses an increased risk of inadvertent damage to other adjacent struc-

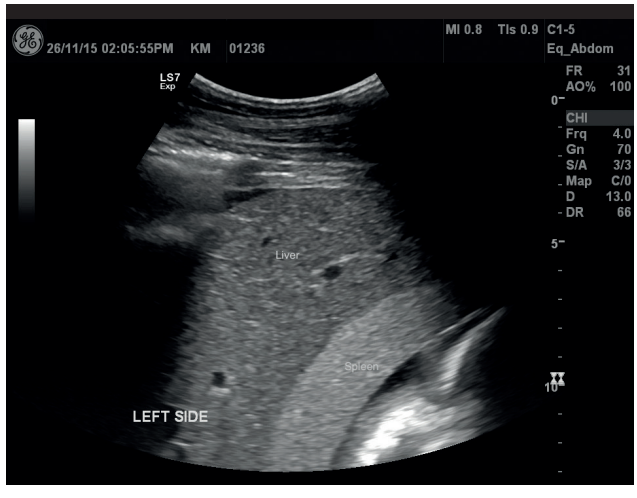

Figure 2. Normal liver (left) adjacent to spleen, illustrating relative hypoechogenicity of liver tissue.

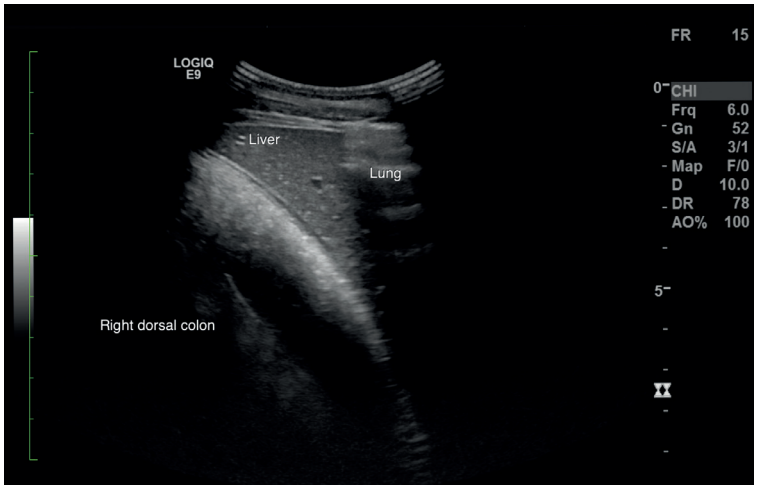

Figure 1. Normal liver adjacent to right dorsal colon and partially obscured by lung.

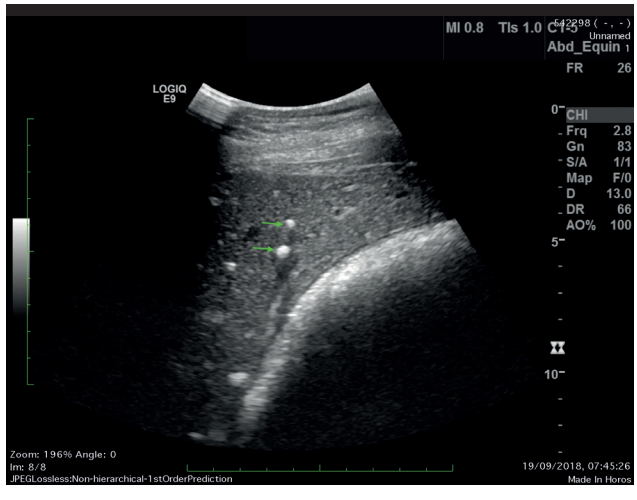

Figure 3. 'Starry sky' liver.

tures such as colon, lung or kidney, and cannot be recommended (Sammons et al, 2014). Ultrasound guided or ultrasound assisted biopsy is a fairly straightforward procedure and is described in detail in *Box 1*. Samples should be submitted for both culture and histopathology. Biopsy has poor sensitivity for very focal disease processes, as only a small amount of liver is analysed. Samples can be obtained from multiple sites to increase diagnostic accuracy in these cases. Biopsy should be carefully considered if a liver abscess

Box 1. How to perform a percutaneous liver biopsy in the horse

Equipment needed (*Figure 3*): 14 gauge Trucut biopsy needle; 10 ml local anaesthetic, sterile 10 cm X10 cm swabs; no. 11 or no. 15 scalpel blade; staple gun or suture material; two sterile sample pots (plain and formalin).

Liver biopsies are traditionally obtained from the right side of the abdomen. However, in older horses the right lobe atrophies and liver may only be visualised on the left. Biopsies from the left side should always be undertaken with ultrasound guidance to avoid risk of inadvertent penetration of the heart or spleen, and may be associated with a higher risk of colic (Divers, 2015). It is not recommended to perform any liver biopsy with a blinded technique. Biopsy may be performed ultrasound guided, where the biopsy tool is visualised as it penetrates the liver. Alternatively ultrasound assisted biopsy involves identification of a suitable site using ultrasound. Depth from skin surface to liver parenchyma is measured. The ultrasound machine is then not used during the procedure. The authors' preference is for ultrasound guided biopsy.

- Sedate and restrain the horse
- Administer flunixin meglumine (1.1 mg/kg intravenously) or other non-steroidal anti-inflammatory of choice
- If performing ultrasound assisted biopsy, identify the appropriate site and measure depth to the liver parenchyma. Use marker pen/correction fluid/staples at two sites outside the sterile field (dorsal and cranial to the ultrasound probe). The needle will be inserted at the intersection of imaginary lines drawn from these two markers (*Figure 4*).
- Clip and aseptically prepare the site
- Infiltrate 10 ml local anaesthetic under the skin, extending to parietal peritoneum (use a 21G 1.5 inch needle to achieve this)
- Make a small stab incision using a no. 11 or no. 15 blade
- Avoid the caudal aspect of the rib where blood vessels and nerves are situated
- Prepare the biopsy tool
- If performing ultrasound guided biopsy, the needle is passed dorsal to the ultrasound probe and is visualised throughout the procedure.
- Advance the biopsy needle through the incision to the level of the parietal peritoneum
- Wait for expiration then advance the needle to the desired depth in liver. Directing the needle slightly cranially may facilitate sampling at maximum liver thickness
- Advance and fire the biopsy tool swiftly, then withdraw.
- Examine the sample — several attempts may be required to obtain a suitable sample
- Place first sample in a plain pot for culture
- Use a small needle to dislodge the other tissue samples into formalin, to minimise any tissue damage
- Obtain 2–3 samples to allow a sufficient number of portals to be submitted for analysis
- The procedure may be repeated at a second suitable site to get representative samples if a focal process is suspected
- Close the skin incision(s) using a staple or suture or leave open and cover with a light dressing
- The biopsy site and adjacent areas can be briefly assessed ultrasonographically following the procedure, to check for the presence of haemorrhage
- The horse should be checked regularly, including heart rate, for several hours afterwards.

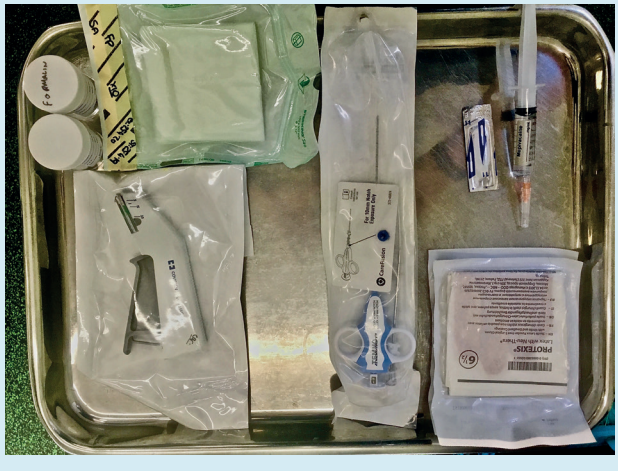

Figure 4. Equipment for liver biopsy.

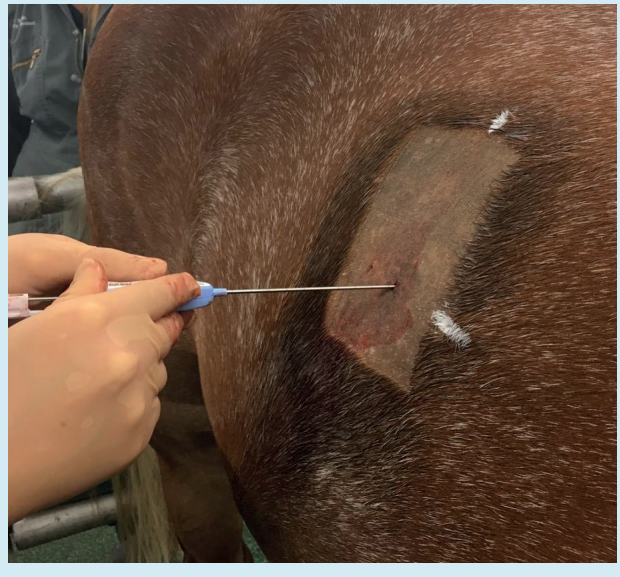

Figure 5. Ultrasound assisted liver biopsy. The white marks on the horse's coat just outside the shaved area are the guidance marks placed during the preparatory ultrasound. The biopsy needle should be pre-marked for the correct depth.

Table 1. Durham Score for liver biopsy

Finding	Absent	Mild	Moderate	Severe
Biliary hyperplasia	0	0	2	4
Haemosiderin accumulation	0	0	0	2
Inflammatory infiltrate	0	0	1	2
Irreversible cytopathology	0	1	2	2
Fibrosis	0	0	2	4

From Durham et al (2003)

is suspected, and the use of ultrasound guidance is imperative to avoid abscess rupture in such cases.

The liver has a limited variety of responses to disease, therefore biopsy findings may not reveal a clear underlying cause in many cases. Early and probably reversible changes include biliary hyperplasia and lipid accumulation. Necrosis of hepatocytes is suggestive of a more recent insult. Following cell death, hepatocytes either regenerate or are replaced by fibrotic tissue. Fibrosis often follows chronic hypoxia, inflammation or exposure to trauma or toxins. Chronic disease with extensive loss of hepatic parenchyma and fibrosis, particularly if it bridges between portals, indicates a poor prognosis. Cirrhosis represents an end-stage process, with fibrosis, nodular regeneration and biliary hyperplasia (Durham et al, 2003).

A scoring system described by Durham et al (2003) is in widespread clinical use and has proven useful for determining prognosis (Dunkel et al, 2015). Based on the presence or absence of five histological characteristics, an overall score from 0–14 is assigned (*Table 1*). Horses with a score of 0–1 were equally likely to survive to 6 months, with a combined mortality of 4%. A score of 2–6 resulted in a mortality of 33% and a 12-fold increased risk of non-survival at 6 months compared to horses with score of zero. A score of 7–14 had a combined mortality of 86% and a 46-fold increased risk of non-survival compared to horses with a score of 0 (Durham et al, 2003). In subclinical cases, mild findings and low scores are not uncommon.

Conclusions

Early-stage liver disease can be challenging to recognise, but once it is suspected, making a diagnosis is relatively straightforward using a combination of serum biochemistry, ultrasound evaluation and liver biopsy. The latter is crucial for determining prognosis and guiding therapeutics, and may identify aetiology in some cases. EQ

Conflict of interest: no conflict.

KEY POINTS

- Liver disease in adult horses is often subclinical or associated with vague, non-specific signs.
- Serum biochemistry is an important diagnostic tool and should include indicators of liver function such as serum bile acids.
- Mild increases in GGT without elevation of other liver enzymes and with normal liver function is commonly identified on serum biochemistry.
- Biopsy may not always identify the underlying cause but provides important information regarding the nature of the disease and associated prognosis.
- Histopathological changes such as severe biliary hyperplasia and bridging fibrosis carry a poorer prognosis..

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