

## INVESTIGATING LIVER DISEASE

### 1. SERUM ENZYMES

Increased serum concentrations of several intracellular enzymes have been reported to be useful in establishing the diagnosis and prognosis of hepatopathies in the horse. Where cases of liver disease are diagnosed it is worth considering screening liver enzymes in herd mates to establish if any others are suffering from subclinical liver disease. In establishing likely causes i.e. toxins in a common feed source, it can also be helpful to test horses that are kept on the same property but subject to different feeding and management.

#### **Alkaline phosphatase (AP)**

Increased serum AP concentration has the strongest association with failure to survive of any enzymes although increased serum AP concentration is neither consistently increased in liver disease nor liver specific. In addition to hepatobiliary sources, serum AP is known to be derived from bone, intestine, inflammatory cells and placenta and these possible sources should be considered in interpretation of increased serum AP concentrations.

#### **Gamma Glutamyl Transferase (gGT)**

Although mild to moderate increases in serum gGT (e.g. up to 100 iu/L) are of limited diagnostic or prognostic value, it is nevertheless very unusual to find significant hepatopathy in horses in the absence of increased serum gGT. Additionally, marked increases in serum gGT concentration (eg. >400 iu/L) are associated with a poor prognosis. Modest increases in serum concentration of gGT should be interpreted with great caution as examination of liver biopsy specimens in such cases often fails to reveal significant underlying liver disease. The pancreas, or even kidneys, could potentially be the source of increased serum gGT in the absence of hepatopathy although renally-derived gGT is widely accepted to appear in urine and not serum. Increases in serum concentrations of liver-derived gGT may result from insults too minor to result in detectable histopathology. For example, horses with intestinal disease and particularly right dorsal displacement of the large colon are often reported with increased gGT perhaps as a result of direct pressure applied to the liver by the distended and heavy colon. Furthermore, cases of liver disease are not infrequently seen where increasing concentrations of serum gGT may be noted despite clinical evidence of improvement of the hepatopathy, perhaps as a consequence of reparative processes or biliary hyperplasia leading to increased serum gGT.

#### **Aspartate Aminotransferase (AST)**

AST is derived from widespread tissue sources and has low specificity for liver disease although in the majority of liver disease cases it will be increased.

#### **Glutamate Dehydrogenase (GLDH)**

Although serum GLDH is entirely derived from the liver, it only has moderate specificity for liver disease probably due to fairly mild and innocuous hepatic insults resulting in increased serum GLDH concentrations. Its relatively short serum half-life might suggest an association between GLDH levels and the currently active degree of hepatic insult. The prognostic usefulness of GLDH is debatable and very high values may be encountered in horses that recover uneventfully.

### 2. SERUM BIOCHEMICAL EVALUATION OF HEPATIC FUNCTION

Serum concentrations of several biochemical substances have been reported to reflect the capability of the liver to perform its normal functions. These are primarily endogenous and exogenous substances that should either be eliminated or synthesised by the liver. They include various amino acids, ammonia (NH<sub>3</sub>), bile acids, bilirubin (total (Tbil) and direct (d Bil)), fibrinogen, globulins, glucose and urea.

#### **Serum globulins**

Hyperglobulinaemia is a common finding in association with hepatic insufficiency probably resulting from systemic immunostimulation by intestinal-derived antigenic material following loss of the protective barrier of Kupffer cells in the liver. When increased serum globulins are found in association with other clinicopathological indicators of liver disease, this is a strong indication that there has been a considerable liver insult and the magnitude of the increase in serum globulin concentration has prognostic relevance. Serum globulin concentrations greater than 45 g/L are concerning and values as high as 60-70 g/L are occasionally seen and warrant a guarded prognosis.

### Serum bile acids

The main limitation of the usefulness of serum bile acid estimation is that liver disease must be quite severe before increased concentrations are detected and most liver disease cases will be found to have normal serum bile acid concentration at the time of initial presentation. Anorexia and inappetence can increase serum bile acids as high as 20-30 mmol/L in the absence of liver disease. Hepatopathy cases with serum bile acid concentrations greater than 20 mmol/L are less likely to survive than those with lower values and chronic cases with bile acid concentrations above 100 mmol/L are almost invariably fatal.

### Serum albumin

Although albumin is synthesised by the liver, it has a long serum half-life hence marked hypoalbuminaemia is uncommon in equine liver disease. Serum albumin concentrations below 20 g/L are very rarely encountered even in severe hepatopathies.

### Bilirubin

Failure of the liver to take up, conjugate and excrete bilirubin may lead to increased serum concentrations of unconjugated and/or conjugated bilirubin. Anorexia and haemolysis are additional causes of unconjugated hyperbilirubinaemia. Horses may have serum unconjugated bilirubin concentrations greater than 200 mmol/L due to anorexia alone although more typically values of less than 150 mmol/L are expected. The magnitude of increased unconjugated bilirubin concentrations associated with acute haemolytic disease is very variable but can be greater than 500 mmol/L. The majority of equine liver disease cases have normal or only moderate increases in serum bilirubin concentration (typically 50-150 mmol/L) and the unconjugated fraction usually greatly exceeds the conjugated fraction. Cases of liver disease in the horse in which serum conjugated bilirubin represents greater than 25% of total bilirubin are likely to have obstruction of the biliary tract.

### Urea and creatinine

Low serum urea concentrations have been recognised previously in association with liver failure and have been suggested to indicate reduced hepatic synthesis of urea from ammonia. Although the majority of equine hepatopathy cases have normal serum urea concentrations, decreased serum urea is associated with more severe hepatopathies and has prognostic relevance.

### Blood clotting times

Hepatic insufficiency is associated with a decrease in the synthesis and function of the majority of procoagulant, anticoagulant and fibrinolytic proteins in addition to reduced platelet numbers and function. Despite the complexities of effects on individual proteins, the net effect of hepatic failure on haemostasis is invariably impairment of coagulation as determined by prolonged APTT and PT, although clinical evidence of coagulopathy is less commonly seen than is clinicopathological evidence of coagulopathy in horses with hepatic insufficiency. The incidence of bleeding disorders associated with performing a liver biopsy is very low and it is not considered necessary to check clotting times prior to performing the procedure.

### Glucose

Despite the central gluconeogenic role of the liver, plasma glucose typically remains within normal limits in adult horses with hepatopathy. Hypoglycaemia is more common in foals with hepatopathy.

### Ammonia

Although plasma ammonia concentration is increased in nearly all cases of hepatic encephalopathy, the concentration does not necessarily correlate with severity of the disease. This apparent paradox may be explained by increased permeability of the blood brain barrier to ammonia in cases of hepatic encephalopathy. Ammonia has to be assayed within minutes and is therefore not offered via our referral laboratory.

Dynamic testing of liver function has been examined in horses using exogenous agents including bromosulphothalein, indocyanine green and radiopharmaceuticals although none appear to be widely used due to limited availability and complexity in contrast to other simple tests of hepatic function.

### 3. LIVER BIOPSY

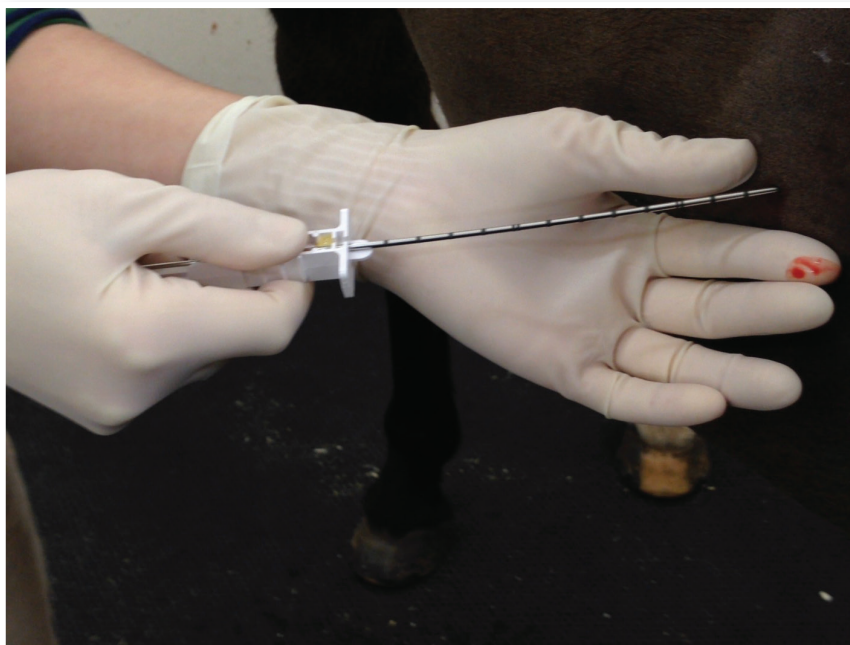
There are three fundamental aims of the investigation of cases of suspected liver disease:

1. To differentiate subjects genuinely suffering from liver disease from those which are not.
2. To determine the type of liver disease and therefore select specific therapy.
3. To determine prognosis.

If liver disease is suspected on the basis of preliminary non-invasive tests then liver biopsy remains the 'gold-standard' technique by which to address these key questions.

Transabdominal ultrasonography should be used to guide liver biopsy and a site is usually chosen based on thickness of imaged hepatic tissue, absence of large vessels and, occasionally, focal presence of hepatic tissue with an abnormal ultrasonographic appearance. The widespread availability of diagnostic ultrasound makes the ongoing use of unguided biopsy techniques questionable.

Adverse effects are very rare but include haemorrhage, colic, peritonitis, pleuritis and pneumothorax. When the technique is performed under ultrasonographic guidance it is extremely safe. Guides are available for many transducers which fix the biopsy needle in the plane of the ultrasonographic image and may facilitate the procedure. However, some clinicians choose to operate the transducer and biopsy needle separately from each other. A quarter of human patients report some degree of abdominal pain following liver biopsy and therefore routine systemic analgesia is recommended in horses.



Performing a Liver Biopsy
<ul style="list-style-type: none"> <li>The subject is sedated.</li> <li>Biopsy site and transducer are prepared for a sterile procedure.</li> <li>10 mL local anaesthetic is infiltrated subcutaneously and through the intercostal muscles to the parietal peritoneum using a 21 g 11/2" needle.</li> <li>A small stab incision is made through the skin using a no.15 (or 11) scalpel blade.</li> <li>A small amount of sterile coupling gel or alcohol is applied to the skin/transducer.</li> <li>A 14 gauge 16 to 20 cm biopsy needle is advanced perpendicularly to the skin into liver and the biopsy is collected.</li> <li>The procedure is repeated if a suitable biopsy specimen is not obtained (2 or 3 attempts sometimes required).</li> <li>Biopsy specimens are placed in 10% neutral buffered formalin for histopathologic examination and/or plain sterile containers for bacteriologic culture.</li> <li>Samples from at least 2 separate sites are preferable.</li> <li>Topical antiseptic spray is applied to the skin incision.</li> <li>A single dose of 2 mg/kg phenylbutazone iv is administered.</li> </ul>

### Interpretation

Marked periportal and bridging fibrosis have frequently been regarded as poor prognostic indicators although long term survival of cases with severe periportal and bridging fibrosis has been reported suggesting other factors are also important. In one study a scoring system was developed in order to attempt to attribute a prognostically useful broad comparative index of histopathologic severity (Table 1). This results in a prognostic biopsy score between 0 (best prognosis) and 14 (worst prognosis). Biopsy scores from 73 cases showed a strong and statistically significant association with survival and survival times. As a general rule horses with biopsy scores of 0-2 will survive, horses with scores above 8 will die and those in between may go either way and require treatment.

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

Table 1. Prognostic biopsy scoring system. Each individual parameter is scored and the total calculated.

### FURTHER READING:

Durham, A.E. *et al.* (2003) An evaluation of diagnostic data in comparison to the results of liver biopsies in mature horses. *Equine Vet J* 35, 554-559.

Durham, A.E. *et al.* (2003) Development and application of a scoring system for prognostic evaluation of equine liver biopsies. *Equine Vet J* 35, 534-540.

Durham, A.E. *et al.* (2003) Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. *Equine Vet J* 35, 542-547.

Rendle, D. (2010) Liver biopsy in horses. *In Practice* 32, 300-305.