

Use of Laboratory Testing to Diagnose Liver and Biliary Dysfunction in the Horse

Katuska Satué Ambrojo, Juan Carlos Gardon Poggi, Ana Muñoz Juzado

Katuska Satué Ambrojo, Department of Animal Medicine and Surgery, Cardenal Herrera University, Spain

Juan Carlos Gardon Poggi, Department of Experimental Sciences and Mathematics, Catholic University of Valencia "San Vicente Mártir", Spain

Ana Muñoz Juzado, Department of Animal Medicine and Surgery, Equine Sport Medicine Center, CEMEDE, University of Córdoba, Spain

Correspondence to: Katy Satué, Department of Animal Medicine and Surgery, Faculty of Veterinary, CEU-Cardenal Herrera University, Tirant lo Blanc, 7, 46115, Alfara del Patriarca, Valencia, Spain. ksatue@uch.ceu.es

Telephone: +34-961369000

Fax: +34-961-39-52-72

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ABSTRACT

Laboratory tests commonly used for detection or confirmation of equine liver disease comprise the measurement of a series of enzymatic parameters as serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ -glutamyl transferase (GGT), glutamate dehydrogenase (GLDH) and sorbitol dehydrogenase (SDH) and biochemical measurements such as proteins, globulins and albumin, bile acids, triglycerides, cholesterol, bilirubin, urea, ammonia, and glucose. Complementary tests might help to categorize a wide spectrum of liver diseases as they reflect the different functions of the liver, and excretion of anions (bilirubin), hepatocellular integrity (enzymes), gall bladder function and free flow (bilirubin and ALP) and protein synthesis. They could indicate the potential cause of liver disease, assess the severity of damage, and help to formulate the prognosis and to evaluate the effectiveness of treatment. Nevertheless, some diseases can be diagnosed more specifically using other techniques diagnostic such as liver biopsy or ultrasound, as well as in many asymptomatic patients. Throughout this review, we will address the interpretation of the main laboratory tests used to detect lesions and to evaluate the function of the liver in the horse.

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Key words: Biochemistry; Enzymes; Horse; Laboratory diagnosis; Liver

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INTRODUCTION

The liver plays an important role in the equine digestive, endocrine, coagulation, and immune systems. Its primary functions are detoxification, synthesis of proteins and biochemical products necessary for digestion. More specifically, the liver has the following physiological functions: protein synthesis (90% of the plasma proteins, such as albumin, factors involved in coagulation and fibrinolysis, transport proteins as transferrin, and acute phase proteins, such as fibrinogen, α and β -globulins, serum amyloid A, C reactive protein...), detoxification of endogenous products (liver is responsible for urea production from ammonia) and exogenous products and toxic drugs (mainly cytochrome p450). In addition, the liver acts in the synthesis and storage of carbohydrates (gluconeogenesis), lipid metabolism (responsible for esterifying free fatty acids into triglycerides for export to other tissues in form of VLDLs or LDLs). The liver plays a vital role in bile acid excretion allowing cholesterol and phospholipids metabolism and it is essential for the absorption of lipids and fat-soluble substances (vitamins A, D, E and K). Also, in the fetus the liver has an important hematopoietic function, and therefore, it can help in the production of erythrocytes under haemolytic, hemorrhagic or hypoplastic-aplastic conditions of the bone marrow^[1]. This organ has an important functional reserve, and as a consequence, it is able to keep most of its functions in face of a damage or disease. However, a highly damaged liver can be a very serious problem^[2].

According to Divers^[3,4], horses and foals are most likely to develop liver damage or disease when they have a pre-existing septic, hypoxic, neoplastic, toxic, or metabolic condition. Although liver disease might be common in horses, they rarely experience liver failure. When they do, however, it is very important to diagnose and treat them quickly and appropriately as this complex condition can be fatal. Indeed, the progression from liver disease to failure is rare, but many causes of liver failure have been described. Toxic causes, such as plants and fungal toxins (ragwort or *Senecio jacobea*, alsike clover, panicum grasses, *Cassia* spp, plants with pyrrolizidine

alkaloids, aflatoxins^[5,6,7,8] are commonly reported as causes of liver dysfunction in horses. Infectious and non-infectious causes of cholangiohepatitis, such as Tyzzer's disease (*Clostridium piliforme*), Theiler's disease or serum hepatitis, cholelithiasis and neoplasias have been also well described^[9,10,11,12,13]. Other less common etiologies of liver diseases in horses are of metabolic origin, such as hepatic lipidosis, physical obstructive causes (right dorsal colon displacement with obstruction of the bile ducts, hepatic torsion, and portal vein thrombosis) and sporadic toxic injuries^[14].

It is important to differentiate between the concepts of liver disease and liver failure. The liver is an organ with an extremely important reserve function and therefore, it requires a loss of at least 60-80% of the organic mass to show signs of liver failure. Such differentiation is relevant because some parameters of liver function such as albumin are affected only when the liver is insufficient and, therefore, they are not sensitive to detect incipient stages of the disease^[15,16].

Evidence of liver disease is often identified when animals are blood tested for a reason other than suspected liver disease, typically due to non-specific clinical signs such as mild weight loss, malaise/lethargy, mild colic or poor performance. The reported clinical signs in animals with confirmed liver disease include depression/lethargy (68%), weight loss (50%), anorexia (56%), colic (50%), photosensitization (16%), diarrhea, icterus (42%) and coagulopathy (10%), pruritus, fever, coronitis and encephalopathy^[10,17,18]. In those horses presenting with neurological manifestations, the main clinical signs are depression (68%), yawning, blindness, disorientation, ataxia, compulsive wandering, head pressing, circling, gastric impaction and bilateral laryngeal paralysis (14%)^[19,20].

The predominant signs of a horse experiencing acute or sudden liver failure are neurologic deficits and icterus. Horses suffering from chronic (ongoing) liver disease that is heading towards failure are likely to lose body weight and have photosensitization. In both cases gastric impactions and bilateral laryngeal paralysis have been noted. Liver failure caused mostly by abnormalities in the biliary system will present with marked icterus, photosensitivity, and colic, probably because of an obstruction in the bile duct and possible enlargement of the liver. These horses do not commonly have neurologic deficits. Additionally, some very specific causes of liver failure are accompanied by very specific clinical signs. For example, cholangiohepatitis will produce fever in horses^[9,10], hepatic lipidosis will produce ventral edema^[14,21], and right dorsal colon displacement will cause abdominal distention and abnormally tight colonic bands felt on rectal palpation^[3,4]. In general, clinical signs of liver disease are not prognostically helpful unless severe because a large proportion of the liver mass (60-70%) must be damaged before function is affected^[22].

The diagnosis of liver disease is based on abnormalities in serum enzymes that are partly or exclusively associated with cells within the liver parenchyma-hepatocytes and/or epithelial cells lining the biliary tree and some analytes that assess liver functionality. Clinical signs of liver disease are often vague although some clinical signs are highly suggestive of hepatopathy. Results of noninvasive tests of liver disease do not always correlate with the degree of hepatic disease and with the outcome of the case. Sub-clinical liver disease is very common, particularly in groups of horses overgrazing poor quality, environmentally stressed pastures or being fed preserved forage taken from such pastures^[23,24].

ANALYTICAL STUDY OF HEPATIC AND BILE FUNCTION IN HORSES

Biochemical analysis is very important in the diagnosis of liver diseases since that the results can be helpful in narrowing the differential diagnosis for the liver failure and, when evaluated over time, can help predict prognosis. These biochemical analyses are used to detect liver specific enzymes and metabolites in the bloodstream, which, if abnormally high or low, are indicative of liver disease and sometimes hepatic failure. Tests performed on blood samples with the intention of investigating liver disease may be subdivided into serum enzymes and indicators of residual hepatic functional capacity^[4].

Enzymes occur normally in the cytoplasm (AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ALT, alanine aminotransferase), mitochondria (GLDH, glutamate dehydrogenase and AST, aspartate aminotransferase), nucleus, or membranes (ALP alkaline phosphatase; GGT, γ -glutamyl transferase) of body cells, where they catalyze specific reactions. The distribution of these enzymes is markedly different among organs, which explains the variation in organ and tissue specificities. Generally, increased plasma enzyme concentrations indicate recent organ damage rather than decreased organ function. Increased enzyme production has been reported in cholestatic liver disease in horses (ALP and GGT). Baseline activity of an enzyme in plasma or serum is generally a reflection of the amount and turnover of the tissue that contains this enzyme. In chronic liver diseases with severe fibrosis and a reduction in the number of functional hepatocytes, plasma/serum activities of liver enzymes may be within normal limits. The increase of a particular enzyme also depends on factors such as its rate of release, rate of production, and rate of clearance from plasma. Cytoplasmatic enzymes will be released early in cell degeneration, whereas mitochondrial enzymes will be released after advanced cell necrosis. Enzymes with high tissue concentrations but with short elimination half-life are of limited value in clinical enzymology because of their rapid disappearance from plasma^[25].

Haematology is rarely helpful but leukopenia and neutropenia are common findings^[26]. In one case of cholelithiasis associated with recurrent colic in a Thoroughbred mare leukocytosis was found^[10], as well as in other hepatic diseases^[12]. Erythrocytosis associated with a hepatoblastoma in a young female horse has been described^[12,27]. Acute phase proteins may support a diagnosis of inflammatory or infective liver disease^[28].

Increased serum concentrations of several intracellular enzymes have been reported to be useful in establishing the diagnosis and prognosis of the equine hepatopathies. Serum biochemistry can identify liver injury and liver dysfunction independently. Elevations in ALP, AST, LDH, GGT, GLDH and sorbitol dehydrogenase (SDH) all indicate liver injury with GGT, GLDH and SDH being liver specific. Experimental studies have suggested a primarily biliary source for GGT, hepatocellular sources for arginase, ALT, AST, GLDH, IDH (iditol dehydrogenase) and LDH and both biliary and hepatocellular sources for ALP^[23,24,29]. The main parameters indicative of altered hepatic functionality are bile acids, triglycerides, bilirubin, urea and glucose. From the clinic point of view of the course of liver or biliary disease, AST and ALP are indicative of chronic course, while SDH, GGT and GLDH indicates acute course^[25].

PARAMETERS INDICATIVE OF LIVER INJURY GAMMA GLUTAMYL TRANSFERASE

GGT is a membrane-bound enzyme located on the external surface of cells and is bound to the cell membrane via a hydrophobic transmembrane peptide. GGT is located on the luminal surface of the proximal tubular cells of the kidney where it is released into urine

during tubular injury. In the pancreas, GGT is located on the luminal surface of cells lining the acini and pancreatic ducts. However, increases of serum GGT activity are not generally associated with primary injury to the pancreas or kidney^[25].

In the liver, GGT is specifically found in the canalicular surfaces of the hepatocytes and in the biliary epithelial cells. The location of GGT in liver is of considerable interest because the liver likely contributes most if not all of the serum GGT activity^[25,30]. Following injury, the mean average half life of equine GGT is 3 days^[31].

Although mild to moderate increases in serum GGT 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 are associated with a poor prognosis. The pancreas, or even kidneys, could potentially be the source of increased serum GGT in the absence of hepatopathy although GGT derived from renal disease is accepted to appear in urine but not serum^[32,33]. Furthermore, cases of liver disease are not infrequently seen with increasing concentrations of serum GGT despite clinical evidence of improvement of the hepatopathy, perhaps as a consequence of reparative processes or biliary hyperplasia leading to increased serum GGT^[23,29].

In a retrospective study of 50 cases of hepatic disease in horses, of the serum chemistry parameters evaluated, only serum GGT activity increased in all cases^[34]. Similarly, serum GGT activity showed 75% sensitivity and 90% specificity for detecting subclinical liver disease in horses exposed to pyrrolizidine alkaloids, whereas serum ALP activity showed only 58% sensitivity^[35]. Serum GGT activity was shown to be the most sensitive serum enzyme for detection of hepatic injury secondary to proximal enteritis in horses^[18]. Increases in serum GGT activity in numerous cases of plant-related hepatotoxicity in horses have also been reported^[35]. Indeed, elevations of GGT activity are related with inflammation or destruction of the epithelium of bile canaliculi and secondary cholestatic hepatocyte swelling^[36]. In cases of biliary obstructions, large increases have been found, with values up to 1670 IU/L^[37]. 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^[38]. Finally, GGT is a highly sensitive and specific indicator of cholestatic biliary disease, acute hepatocellular damage (hepatocyte necrosis) and primary and secondary liver tumours.

SORBITOL DEHYDROGENASE

SDH also known as iditol dehydrogenase or IDH, catalyzes the reaction of sorbitol + NAD to fructose + NADH. SDH remains stable for 5 hours at room at environment temperature, 5 hours at refrigerated and 48 h frozen^[39]. SDH is a liver-specific enzyme in horses. It is considered a good indicator of active hepatocellular disease, which increases in the moderate and severe cases of intra- and extrahepatic cholestasis, respectively. SDH has a short half-life and should be determined within the first 6 hours of the sample extracted^[25].

ASPARTATE AMINOTRANSFERASE

AST (or glutamic oxaloacetic transaminase; GOT) catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. Although AST is mainly located in the cytosol it is also appears in high concentrations in the mitochondria. AST is not specific of hepatic diseases because is also found in striated muscle, intestine, kidney and erythrocytes. 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. The half-life has been reported to be as long as 7 to 8 days in horses^[25].

Increased serum AST activity is observed with both reversible and irreversible injury to hepatocytes and can be seen following hepatocellular injury and cholestasis. Likewise, serum AST is increased following myocyte injury. In either case, the definitive disease process cannot be identified, only that cellular injury in muscle or liver has occurred. Because serum AST activity cannot differentiate between hepatocellular or myocyte injury, further testing is often required using organ-specific enzymes such as SDH or creatine kinase (CK). Markedly increased serum AST and SDH suggest acute or active hepatocellular injury, and markedly increased serum AST with modest to moderate SDH activity suggests chronic hepatic injury or recovery from acute liver injury. An increased serum AST together with CK is a clear indication of muscle damage. The sensitivity of serum AST activity in horses has been reported as 72% for hepatic necrosis and 100% for hepatic lipidosis^[40].

For a correct diagnosis, determination of AST activity should be complemented with other indicators of damage and liver dysfunction, such as SDH, bile acids, bilirubin, and GGT among other^[25]. Nevertheless, serum GLDH activity was shown to significantly increase in acute, subacute, and chronic grass sickness in horses^[41]. However, serum GDH activity was found to be highly variable in ponies exposed to pyrrolizidine alkaloids, suggesting that GLDH activity may only be diagnostically useful in the acute stages of liver injury^[42].

SERUM ALKALINE PHOSPHATASE

Although many tissues or cell types have some ALP activity, cells from liver, bone, kidney, intestinal mucosa, and placenta have the greatest ALP activity on a per gram of tissue basis with intestinal mucosa having the most^[43]. Cholestasis is the most common cause of significant increases in serum of ALP in horses^[25]. ALP increased after 48 h of liver damage and it is usually higher in cholestasis than in hepatocellular damage. Its increase may occur by the administration of some drugs (phenobarbital, corticosteroids and others ...). Care needs to be taken in pregnancy, ALP changes are due to placental origin of this enzyme, and in growing animals in which normal values are 2 or 3 times over the reference values in adults because of increased bone turnover associated to physical growth^[1].

GLUTAMATE DEHYDROGENASE

GLDH is a mitochondrial enzyme that catalyzes the removal of hydrogen from L-glutamate to form the corresponding ketimine acid that then undergoes spontaneous hydrolysis to 2-oxoglutarate. The liver has by far the highest concentration of GLDH activity^[44]. Lesser amounts are found in the kidney and the small intestine, where the GLDH activity is located in the proximal and distal tubular epithelial cells and in the mucosal epithelial cells, respectively. The GLDH activity of nonhepatic tissues is relatively small compared to that found in liver, where GLDH is concentrated in the central areas of the lobule.

In horses, increases in serum GLDH activity are considered liver specific. Its relatively short serum half-life of 14 hours might suggest an association between GLDH levels and the currently active degree of hepatic insult in response to ischemia and hepatic toxicity^[23,29,34,41]. 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.

This parameter should be interpreted with caution in foals, as the activity of this enzyme is increased in infants or young foals in the absence of liver disease^[25].

The sensitivity of GLDH activity for detection of hepatic necrosis, hepatic lipidosis, and hepatic cirrhosis was 78%, 86%, and 44%, respectively^[40]. The sensitivity was higher than that of SDH and comparable to that observed of serum AST activity^[23].

PARAMETERS INDICATIVE OF HEPATIC FUNCTIONALITY

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 synthesized by the liver. They include proteins, albumin and globulins, ammonia, bile acids, total and direct bilirubin, urea, creatinine, glucose and fibrinogen. Elevations in ammonia, serum bile acids (SBA), bilirubin, globulin and clotting times and reductions in albumin, urea and fibrinogen are suggestive of significant liver dysfunction. Serum biochemistry values can be prognostically valuable with significant elevations in GGT, ALP, SBA and globulins and decrease in albumin and urea having some predictive value for survival at 6 months. In general blood tests are very useful in diagnosing liver disease but have limited prognostic value. Some biochemical indices are of some help; significantly elevated globulin (13× death risk at 6 months) and SBA levels (10× death risk at 6 months) and significantly reduced albumin levels (10× death risk at 6 months)^[26].

SERUM ALBUMIN AND GLOBULINS

Although albumin is synthesized by the liver, it has a long serum half-life in horses and as a consequence, marked hypoalbuminaemia is uncommon in equine liver disease. Serum albumin concentrations below 20 g/L are very rarely encountered even in severe hepatopathies^[5,23,29,34]. Albumin concentrations in foals are significantly higher in the early neonatal period, underscoring the importance of using age-matched references when evaluating clinical pathology values during the neonatal period^[2,23].

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^[45,46]. 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^[23].

SERUM BILE ACIDS

SBA are synthesized from cholesterol in the hepatocytes through various pathways. They are mainly conjugated with either glycine or taurine and after, they are excreted into the bile. Bile acids are responsible for the solubilization of dietary fat in preparation for absorption. Upon entry into the lumen of the gastrointestinal tract, the primary bile acids are dehydroxylated by resident bacteria and form secondary bile acids. Subsequently, through enterohepatic circulation, non-degraded primary and secondary bile acids are absorbed from

the small intestine and transported back to the liver via the portal vein for re-excretion in the bile. Approximately 90% of the bile acid pool is restricted to the enterohepatic circulation and is estimated to cycle approximately 40 times a day in ponies^[2,47].

The determination of the serum concentrations is considered a highly sensitive assay in cases of liver dysfunction, and their increase is an excellent indicator of liver failure. It should be noted that increased values can be found due to prolonged fasting (>3 days). The main limitation of the usefulness of SBA 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 SBA concentration at the time of initial presentation. Anorexia can increase SBA as high as 20-30 mmol/L in the absence of liver disease. Hepatopathies with SBA 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^[23,29].

TRIGLYCERIDES

Triglycerides are synthesized as an energy reserve mainly in the adipocytes and hepatocytes. They are fatty acids with 92% to 95% and the glycerol moiety^[3]. Triglycerides are good indicators of liver function, because increased serum concentrations, are associated with lipid accumulation in the liver, interfering with the normal function of the organ^[21,22].

BILIRUBIN

Bilirubin is a bile breakdown product of several heme proteins that is subsequently conjugated in the liver. Assessment of serum direct and indirect bilirubin concentrations is useful for diagnosing liver dysfunction in horses. Increased bilirubin concentrations are associated with hemolysis, hepatocellular disease, cholestasis and physiological causes^[37].

Failure of the liver to take up, conjugate and/or excrete bilirubin may lead to increases in serum unconjugated and/or conjugated bilirubin. Anorexia and hemolysis are additional causes of unconjugated hyperbilirubinemia^[48].

Horses may have serum unconjugated bilirubin concentrations greater than 200 mmol/L because of anorexia alone, although more typically values of less than 150 mmol/L are expected. The magnitude of the increase in unconjugated bilirubin concentrations in acute hemolytic disease is very variable but can be greater than 500 mmol/L^[48]. 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, even though in the horse fasting causes hyperbilirubinemia^[49]. Furthermore, increased unconjugated bilirubin concentrations, which appears in equine liver disease is associated with acute hepatocellular, although not necessarily indicate the presence of liver insufficiency. Similarly, bilirubin values can be within normal limits in animals with severe chronic liver diseases^[50].

UREA AND CREATININE

Urea is synthesized in the liver from ammonia, then is filtered by the glomerulus and reabsorbed passively in part by the renal tubules. Low serum urea concentrations have been recognized 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^[23].

GLUCOSE

Glucose in blood circulation is a source of energy for most of the tissues. It is stored in the liver in the form of glycogen. Therefore, liver has a pivotal function in maintaining normal blood glucose concentrations through degradation and resynthesis of glycogen. Despite the central gluconeogenic role of the liver, plasma glucose typically remains within normal limits in adult horses with hepatopathy, although a decline in glycemia has been described in horses with hepatic necrosis, lipidosis, neoplasia and cirrhosis^[51,52]. A fall in plasma glucose concentration carried a guarded prognosis. Hypoglycaemia is more common in foals with hepatopathy than adult horses^[54].

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^[53]. Ammonia has to be assayed within minutes.

DYNAMIC TESTS OF LIVER FUNCTIONALITY

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. In horses with hepatic necrosis, lipidosis, neoplasia and cirrhosis plasma ammonia concentrations were significantly elevated compared to clinically normal horses, but such changes were not always accompanied by a decline in plasma urea concentration^[51,54].

OTHER PARAMETERS INDICATIVE OF HEPATIC DISEASE

BLOOD COAGULATION 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 hemostasis is invariably an impairment of the coagulation, as determined by prolonged activated partial thromboplastin time (APTT) and prothrombin time (TP), although clinical evidence of coagulopathy is less commonly seen than clinicopathological evidence of coagulopathy in horses with hepatic insufficiency^[55]. 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^[3]. Recently, it has been found that an abnormal coagulation profile is not clearly associated with an increased risk of complications after biopsy in horses with liver failure^[55].

FIBRINOGEN

Fibrinogen is synthesized in the liver, hence, in horses with

liver failure can have a significant decrease in fibrinogen concentrations^[23,29]. However, fibrinogen is an acute-phase protein and it is a widely used marker for diagnosis of acute inflammatory processes. Therefore, it is important to consider if there is a concomitant liver failure when interpreting fibrinogen concentrations in blood^[16].

RATIO between branched aminoacids: aromatic aminoacids

During liver insufficiency, there is an imbalance in plasma aminoacids ratio, between branched chain amino acids (BCAA) and aromatic amino acids (AAA). A decrease in this ratio has been associated with an increased risk of developing hepatic encephalopathy signs, especially if this value falls below 2.5^[8,56,57].

Other laboratorial findings found in hepatic diseases

Finally, in hepatic necrosis, lipidosis, neoplasia and cirrhosis, other factors, such as hypokalaemia, alkalosis, short chain volatile fatty acids and false and true neurotransmitters may be important in the pathogenesis of hepatic coma in the horse^[51].

A significantly poorer prognosis was found in association with increased serum globulins, increased total bile acids, increased ALP, GGT, erythrocytosis, leukocytosis, low serum albumin and urea. An additional significant novel finding of interest is the association between increased plasma fibrinogen and low serum creatinine concentrations with non-survival in cases of liver disease^[23].

Normal values of enzymes and others biochemical parameters used to diagnosis of hepatic disease in the horse and the list of hepatic diseases and laboratory test patterns characteristic are included in table 1 and table 2, respectively.

Table 1 Normal values of enzymes and others biochemical parameters used to diagnosis of hepatic disease in the horse (Kaneko *et al*^[58]).

| Serum/Plasma Enzyme Activities | Reference Range |
|---|-----------------|
| γ-Glutamyl Transferase (Ggt; Ui/L) | 5-20 |
| Sorbitol Dehydrogenase (Sdh; Ui/L) | 2-10 |
| Aspartate Aminotransferase (Ast; Ui/L) | 150-300 |
| Alcaline Phosphatase (Alp; Ui/L) | 120-250 |
| Glutamate Dehydrogenase (Gldh; Ui/L) | 2-10 |
| Other Biochemical Parameters | |
| Albumin (G/Dl) | 2.6-3.7 |
| Globulins (G/Dl) | 2.62-4.04 |
| Serum Bile Acids (Umol/L) | 5.0-28.0 |
| Tryglycerides (Mg/Dl) | 4.0-44.0 |
| Bilirubin (Total) (Mg/Dl) | 1.0-2.0 |
| Bilirubin (Direct) (Mg/Dl) | 0.0-0.4 |
| Bilirubin (Indirect) (Mg/Dl) | 0.2-2.0 |
| Urea (Mg/Dl) | 0.9-1.1 |
| Creatinine (Mg/Dl) | 1.2-1.9 |
| Glucose (Mg/Dl) | 75.0-115.0 |
| Ammonia (µG/Dl) | 13.0-108.0 |
| Blood Clotting Times | |
| Prothrombin Time (Pt; Sec) | 7-9 |
| Activated Partial Thromboplastin Time (Aptt; Sec) | 37-54 |
| Fibrinogen (G/Dl) | 0.2-0.4 |
| Arginase (Ui/L) | 0-14 |

CONCLUSIONS

Although primary and secondary liver diseases are common in horses, liver failure is by far less common in equine medicine. Clinical signs indicative of liver failure are vague and non-specific and therefore, laboratory diagnosis is pivotal for diagnosing this type of disease. The main two groups of markers of liver disease are those indicative of hepatocyte and/or biliary tract injury (liver and cholestatic enzymes) and those indicative of loss of liver functionality. The most useful enzymes to diagnose liver and/or

biliary injury are AST, LDH, SDH, GLDH, ALP, SDH and GGT. The most used markers of liver functionality are blood proteins (both albumin and globulins), bile acids, triglycerides, urea, creatinine,

glucose, bilirubin and ammonia. The measurement of coagulation times and fibrinogen concentrations is important for both diagnosing liver disease and setting a prognosis.

Table 2 Common hepatic pathologies in the horse (Barton^[50]).

| Specific Hepatic Diseases | Laboratory profiles |
|--|---|
| Acute Hepatic Diseases | |
| Theiler's Disease | Bilirubinemia and bilirubinuria usually present. SDH, arginase and AST increased. PT and APTT often are prolonged. Ammonia concentrations are increased. |
| Tyzzler's Disease | SDH, GGT, AST, ALP and LDH-5 are increased. Hyperfibrinogenemia and hypoglycemia. |
| Toxic Hepatopathy | AST and GGT are increased. In iron toxicity: hyperbilirubinemia and prolonged PT, reduced BCAA:AAA ratio and glucose. |
| Hyperlipemia | Hypertriglyceridemia (> 500 mg/dl), bilirubinemia are present. SDH, GGT and SBA are increased. Glucose, BUN and ALB are reduced. |
| Chronic hepatic diseases | |
| Chronic Megalocytic hepatopathy | SDH and AST are increased (early in disease). GGT and ALP are elevated (fibrosis). Bilirubinemia is present. BCAA:AAA ratio is decreased. |
| Chronic Active Hepatitis | SDH and AST are mildly increased and, GGT and ALP are greatly increased. SBA, bilirubinemia and bilirubinuria are present. |
| Cholelithiasis | GGT (> 15 times normal) and SBA are increased. SDH, arginase, AST and LDH-5 are mildly to moderately increased. Hyperbilirubinemia and hyperbilirubinuria are present. PT and APTT often are prolonged. |
| Hepatic Neoplasia | SDH and GGT are elevated. Hyperfibrinogenemia (carcinoma and hepatoblastoma). |
| Congenital abnormalities (bold) | |
| Portosystemic shunt | Ammonia concentrations are increased and BUN is decreased. |
| Biliary atresia | SDH, GGT and conjugated bilirubin are elevated. |

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Peer reviewer: Steven Weinman, MD, PhD, University of Kansas Medical Center, 3901 Rainbow Blvd, MSN 1018, Kansas City KS, 66160, the United States.