Diseases of the liver can be a diagnostic and therapeutic challenge for equine practitioners. Clinical signs of liver dysfunction are often nonspecific and result from abnormalities of metabolism and excretion. The most common etiologies of pasture-associated liver disease are pyrrolizidine alkaloids, panicum grasses, and clover (alsike and red clover). Diagnostic testing is valuable for determining etiology and prognosis as well as for directing appropriate therapy.

1. Introduction

The liver regulates energy metabolism and biotransforms and eliminates foreign substances. Clinical signs of liver dysfunction are often nonspecific and result from abnormalities of metabolism and excretion. Common historical and physical examination abnormalities include icterus, poor body condition, anorexia, lethargy, and intermittent mild colic. Phylloerythrin, a byproduct of bacterial degradation of chlorophyll, may accumulate in blood and tissues, causing photosensitization. Cerebral dysfunction, also known as hepatic encephalopathy, may range from subtle behavioral changes to stupor and coma. Other signs of liver disease include diarrhea, ventral edema, pruritus, polydipsia, bilateral laryngeal paralysis, and injected mucous membranes. Because loss of more than 80% of liver function is necessary for clinical signs to appear, liver disease is often severe and widespread prior to diagnosis. The purpose of this review is to review the most common etiologies of pasture-associated liver disease, diagnostic testing options, and treatment.

2. Etiologies

Chronic megalocytic hepatopathy is the most common cause of chronic hepatic disease in horses in the United States. It is caused by ingestion of pyrrolizidine alkaloid (PA)-containing plants including Crotalaria spp., Senecio spp., Amsinckia spp., Heliotropium europaeum, Echium plantagineum, and Cynoglossum officinal. Toxicity is cumulative over the course of a lifetime; with consumption of 2% to 5% body weight required for induction of liver disease. These plants are generally unpalatable to horses, but palatability increases with weather extremes such as drought or frost. The alkaloid remains toxic despite drying, so horses may become intoxicated by exposure to plants baled into hay. In addition, pastures may become overgrown with the plant, increasing the likelihood of consumption. Toxicity occurs in horses of all ages and breeds, but not all horses on a farm will develop signs of hepatic dysfunction.

Clinical signs of PA toxicity typically develop weeks to months after consumption of sufficient
quantity of the toxic plant and are consistent with chronic hepatic disease. Clinicopathologic abnormalities at the time of diagnosis include but are not limited to increases in \( \gamma \)-glutamyl transferase (GGT), alkaline phosphatase (ALP), and serum bile acids. Serum bile acids concentrations greater than 50 \( \mu \text{mol/L} \) have been associated with an increased risk of nonsurvival. Horses with subclinical PA toxicity may have increased activities of GGT, it is therefore recommended to serially monitor GGT in horses on the same property as a known case.

The alkaloid causes cross-linking of DNA, formation of DNA adducts, and DNA strand breakage. By cross-linking DNA, hepatocytes are incapable of undergoing nuclear division, thus preventing regeneration of parenchyma in the face of cellular damage. This leads to fibrosis of the hepatic parenchyma. The hallmark histologic findings in PA toxicity are therefore megalocytosis (presence of cells with very large cytoplasm and a single nucleus), bridging portal fibrosis, and biliary hyperplasia. Although reports exist of horses surviving PA intoxication, the prognosis is generally poor especially when clinical signs are apparent. No specific treatment exists, but life may be prolonged by appropriate attention to supportive care and nutrition. The disease may be prevented by removal of all PA-containing plants from pasture and ensuring that hay sources are free from PA-containing plants.

Toxicity has been reported from ingestion of alsike clover (\( \text{Trifolium hybridum} \)) or red clover (\( \text{Trifolium pratense} \)). These legumes grow in moist soils and have been cultivated as a forage source, often alongside alfalfa. Horses become exposed either through pasture grazing or consuming hay containing the plant. The toxic principle is unknown, but ingestion of a diet of 20% alsike clover for 2 weeks will induce signs of liver disease. Clinical disease may manifest as acute or chronic hepatic dysfunction. Clinical signs of acute disease are those of hepatic encephalopathy such as lethargy, depression, altered mental status, head pressing, and behavioral changes. Signs of chronic disease include ill thrift, anorexia, icterus, and lethargy. Phototoxic sensitization has been reported frequently with the disease but may represent a separate disease process, as many horses with photosensitization lack liver disease. This disease is distinct from slaframine toxicosis (aka black patch disease or ”slobbers”), which is caused by ingestion of red clover infected with the fungus \( \text{Rhizoctonia leguminocola} \) and characterized by hypersalivation without effects on the liver. It is also important to note that alsike clover and red clover are a different genus from sweet clover (\( \text{Melilotus sp.} \)). Moldy sweet clover consumption would result in clinical signs of coagulopathy.

Laboratory abnormalities associated with clover toxicity include hyperbilirubinemia, increases in liver enzymes, and increases in serum bile acids. Gross postmortem abnormalities include an enlarged liver, and histopathology reveals biliary hyperplasia and periportal fibrosis that may cross the limiting plate. Inflammatory changes, megalocytosis, and bile retention are not features of the disease, and hepatocyte regeneration may be present. Treatment is focused on supportive care and removal of the plant from the premises and from hay. The prognosis is fair, depending on the degree of fibrosis. Regeneration of the parenchyma remains possible, and the liver may regain function.

Hepatic toxicity has also been documented from ingestion of hay containingfall panicum (\( \text{Panicum dichotomiflorum} \)) in multiple horses in a boarding stable and has been reported in horses grazing the grass. Horses naturally intoxicated as well as two horses experimentally intoxicated developed signs of liver disease including icterus, lethargy, anorexia, and weight loss after 2 to 3 weeks of eating the contaminated hay. The most notable laboratory abnormalities included marked increases in the activities of GGT, sorbital dehydrogenase (SDH), ALP, aspartate aminotransferase (AST), and mild to moderate increases in creatine kinase. Marked increases in concentrations of total and unconjugated bilirubin and serum bile acids may also occur. Histopathologic abnormalities were predominately hepatic necrosis with mild hepatocyte vacuolation, fibrosis, and bile duct proliferation. The prognosis is fair to poor, with 5 in 14 horses euthanized because of clinical deterioration. 

3. Diagnostic Testing

Laboratory testing is required for diagnosis of liver disease. Clinicopathologic data, ultrasound examination, and liver biopsy provide the most comprehensive diagnostic utility. The enzymes that are specific for liver in the horse are GGT and SDH. GGT is a microsomal protein present on the surface of epithelial cells of the biliary tract, renal tubules, mammary gland, and pancreatic tissue. An increase in the activity of GGT in serum or plasma is specific for cholestatic disease. Other causes of increases in plasma GGT include pancreatic disease (extremely uncommon) and large colon displacement. The half-life of plasma GGT is 3 days; therefore GGT may remain increased for days to weeks after an acute insult. SDH is a cytosolic enzyme that is released with hepatocellular necrosis or changes in hepatocyte membrane permeability. It is not an inducible enzyme, and increases are specific for hepatocellular disease. The half-life of plasma SDH is 12 to 24 hours, with values returning to normal within days of an acute insult. The stability of SDH at room temperature is poor, with activities remaining stable for only 4 hours. It loses 3.5% activity per day when refrigerated and 1% activity per day when frozen. It is therefore important to measure SDH within hours of blood collection or refrigerate or freeze samples as soon as possible after blood collection.
Other less specific enzymes that suggest liver disease when activities are increased include AST, lactate dehydrogenase (LDH), and ALP. AST is a cytosolic enzyme present in all cells, with the highest activities within skeletal muscle, hepatocytes, cardiac muscle, the gastrointestinal tract, and erythrocytes. LDH is a cytosolic enzyme with cellular distribution similar to that of AST. LDH isoenzyme-5 is present in skeletal muscle and hepatocytes and is more specific for hepatocellular necrosis than overall LDH. ALP is present on the mitochondrial membrane of bone, intestine, kidney, placenta, and leukocytes. Evaluation of all of the aforementioned enzymes should be done in light of other specific indicators of liver disease.

Conjugation and excretion of bilirubin is performed solely within the liver. With liver dysfunction, conjugated (direct reacting), unconjugated (indirect reacting), or both may be increased. Increased plasma concentration of conjugated bilirubin is suggestive of cholestatic disease, whereas increased concentration of unconjugated bilirubin may be caused by anorexia, extravascular hemolysis, and hepatocyte dysfunction.

Measurement of serum bile acids assesses liver function. Bile acids undergo enterohepatic recycling and are removed from the circulation by functional hepatocytes. Increases in bile acids may be caused by poor hepatic perfusion or inability of hepatocyte to take up, conjugate, or excrete bile acids. Fasting is not required for the measurement of serum bile acids in the horse.

Other laboratory abnormalities that may exist in horses with liver disease include hypoalbuminemia, hyperglobulinemia, hypoglycemia, decreased blood urea nitrogen, hypertriglyceridemia, leukocytosis, and hyperfibrinogenemia. Blood ammonia will be increased in horses with hepatic encephalopathy.

In horses with laboratory abnormalities suggestive of liver disease, percutaneous liver biopsy is the sole method of determining a definitive diagnosis and prognosis for the life of the horse. A presumptive diagnosis may be made from history of exposure to a toxic plant, along with clinical signs and laboratory abnormalities. It is recommended to perform the biopsy using ultrasonographic guidance. The majority of the equine liver is present on the right side of the abdomen, caudal to the diaphragm, but a section may be visible in the left cranioventral abdomen. Transabdominal ultrasound enables evaluation of liver size and architecture, determination of the presence of masses or choleliths, and evaluation of blood vessels and the biliary tree. An appropriate site for biopsy may be chosen after a complete ultrasound examination. Coagulation times (prothrombin time and activated partial thromboplastin time) should be performed prior to percutaneous biopsy. One study demonstrated as many as 58% of horses with liver disease have abnormal coagulation times, but only 12.5% of horses had clinicopathologic evidence of hemorrhage, and no horses developed clinically significant hemorrhage following biopsy. Hepatic histopathology consistent with a poor prognosis includes the presence of megalocytes, neoplastic cells, fibrosis, biliary hyperplasia, hemosiderin accumulation, and inflammatory infiltrate, with an increasing degree of each factor worsening the prognosis. Biopsy samples should be submitted for both histopathology and culture.

4. Treatment
Therapy for liver disease is largely supportive and may be directed at an underlying etiology, if known. Other than removing the offending plants, a specific directed therapy for pasture-associated liver disease is not available. If biopsy results reveal severe bridging fibrosis of the liver, treatment is unlikely to provide any benefit to the patient. Therapy may depend on the severity of clinical signs and/or evidence of hepatic failure. Crystalloid fluids (50 ml/kg per day) with supplemental 5% dextrose and potassium may be beneficial for some cases and is indicated in hepatic encephalopathy. A low-protein, high-energy diet may be beneficial. Branched-chain amino acid treatment may decrease the severity of neurologic signs. Lactulose (0.3 mL/kg every 6–12 hours) or neomycin (10 mg/kg every 6 hours) is often administered orally in horses with hepatic encephalopathy to decrease enteric ammonia production but may result in diarrhea in some horses. Additional therapies directed at hepatic encephalopathy are available but are beyond the scope of this review.

Cholangiohepatitis is typically included on the initial list of differential diagnoses; therefore, broad-spectrum antimicrobial therapy is warranted. The authors routinely utilize trimethoprim sulfamethoxazole (20–30 mg/kg orally every 12 hours) as an initial choice if the patient is clinically stable and treatment is initiated before the results of liver biopsy. Penicillin/gentamicin, enrofloxacin, and metronidazole (in combination with the aforementioned choices) may also be utilized. Parenteral antimicrobials may be utilized in a hospital setting or if clinical signs are more severe. Flunixin meglumine (0.5–1 mg/kg) may be administered for its anti-inflammatory and analgesic effects. Pentoxifylline (8.5 mg/kg orally every 12 hours) has anti-inflammatory properties and has been shown to reduce hepatic fibrosis in human patients.

5. Summary
Pasture-associated liver disease is not uncommonly seen in equine practice. Knowledge of the plants that cause hepatic disease will promote early diagnosis and improve outcomes. PAs, panicum grasses, and clover (alsike and red clover) are most commonly implicated. Histopathology may be able to differentiate toxic etiologies, but is most helpful for determining the prognosis, based on the presence or absence of fibrosis. Clinicopathologic testing is
critical for determining hepatic disease; GGT, SDH, and serum bile acid concentration are the most liver-specific.

References