

LIVER DISEASE AND FAILURE IN HORSES

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INTRODUCTION

My interest in equine liver disease was first stimulated by Dr. Robert Whitlock (DVM, PhD) while at UGA (76-79) and UPenn (80-89) and then following my move to Cornell I had the great opportunity to continue my research on equine liver diseases with Dr. Bud Tennant (DVM and Research Fellow Mass. General). Dr. Tennant was a renowned comparative hepatologist who in 2016 was awarded, by the Hepatitis B Foundation, the Baruch S. Blumberg Prize for his research and contributions to the field of Viral Hepatitis.

CAUSES OF LIVER DISEASE AND FAILURE IN HORSES

Liver disease is common in horses and foals suffering from toxic, septic, hypoxic, neoplastic, vascular or metabolic conditions, but progression to liver failure is rare. Previously reported toxic plants causing liver failure in North America include the following: pyrrolizidine alkaloids (PA) (e.g., *Senecio* spp., *Amsinckia* spp.), alsike clover, and *Panicum* grasses (Kleingrass, fall *Panicum*). Most of my experiences with PA toxicity come from my different employments at UC Davis (no one wanted to hire me for very long). This disease is relatively rare in the northeastern U.S. Although PA toxicity is generally a high mortality disease, I will provide information in the presentation on a farm outbreak that had a mostly favorable outcome. Fall *Panicum* toxicity had not been reported until 2006 but we had this on our radar as a possible cause of hepatic failure outbreaks for a couple of years prior to confirming the disease in 2006 (publication by Amy Johnson, J Vet Intern Med. 2006- Amy is now in the internal medicine department at NBC). Although iron toxicity (hemochromatosis) is frequently postulated as a cause of liver failure in horses, it has rarely been documented in adult horses. Based upon experiences and previous published experimental studies, I had formed a belief that iron toxicity was a very rare cause of liver failure in horses. Although adult horses do appear to be relatively resistant to high iron feeds they can develop chronic liver failure from prolonged drinking of high concentration iron water. (Equine Vet J. 2019 May;51(3):304-309). It is well known from studies at NBC and elsewhere that iron containing products should not be administered to neonatal foals prior colostrum or acute hepatic necrosis may occur. The neonatal foal iron toxicity outbreak in the Spring of 83 was certainly an interesting investigation and one of my memories of that study is having a very smart veterinary student helping with the study (Dr. Noah Cohen VMD). Iron toxicity has also been suggested to be the cause of hepatic cirrhosis in 1-5 month foals that had previously received multiple whole blood transfusions as treatment of neonatal isoerythrolysis but this has not been documented nor has successful treatment with deferoxamine been reported in these foals. Documenting iron toxicity is more difficult in horses than in most other species because serum iron and iron saturation can be very high in horses with liver failure due to other causes and horses tend to normally develop significant iron accumulation in the hepatic periportal area (hemosiderosis). Mycotoxins, microcystin (blue green algae) and phosphine ingestion are uncommon causes of equine liver failure. The combination of rifampicin and doxycycline has caused liver failure in foals.

Infectious causes of liver failure in adult horses are most commonly cholangiohepatitis with or without stones and viral hepatitis (Theiler's disease). Less commonly, *Clostridium novyi* secondary to hepatic fluke infection may cause liver failure in adult horses. In foals, infectious

causes of liver failure include; Tyzzer's disease and less commonly *Actinobacillus*, EHV1, *Streptococcus zooepidemicus* and *Leptospira* infections as causes of liver failure. Although Tyzzer's disease which is caused by *Clostridium piliformis* is generally a sporadic and highly fatal acute hepatitis in 7-42 day old foals, it can also occur as outbreak on some large farms. I have helped with the investigation on a couple of these farms and have not figured out the transmission. My first thought was that the mares on the farm may have a high prevalence of fecal shedding of the organism (the age range of the affected foals would coincide with the time that foals commonly ingest their dams fresh feces) but so far, the mares fecal PCR tests have been negative. Another possibility is that another species may be shedding the organism in their feces and contaminating the feed but this has not been documented. The mortality rate is not 100% with Tyzzer's disease as several of these foals have recovered with antimicrobial therapy. Tyzzer's disease in foals should not to be confused with Theiler's disease in adult horses!

Perhaps the biggest breakthroughs in the investigation of equine hepatic disorders has been the discovery of several equine hepatic viruses? There are numerous publications from the United States, Europe and elsewhere describing newly discovered hepatitis viruses in horses. A review of these viruses has recently been summarized by Dr. Joy Tomlinson DVM, who completed a residency at NBC (Vet Clin North Am Equine Pract. 2019;35(2):351-362). In summary two of the initially "so called" hepatitis viruses are not "hepatitis viruses". These are the two equine pegiviruses, initially called equine pegivirus1 (EqPV1) and Theiler's disease associated virus (TDAV). These two RNA viruses have now been proven to not cause liver disease and neither is hepatotropic but instead they have tropism for the bone marrow. We originally thought TDAV was a cause of Theiler's disease, hence it's name, as it was found in association with an outbreak of hepatitis in horses 6 weeks after the horses had been administered an equine origin antitoxin. Blood from the horses was initially investigated using targeted metagenomic next-generation sequencing (mNGS) looking for RNA viruses and the pegivirus was found. After this discovery, a prospective case study on Theiler's disease found no TDAV refuting the hypothesis that TDAV was the cause of Theiler's disease. Two years later, testing with untargeted mNGS provided strong evidence that Theiler's disease is actually caused by a DNA virus. A summary for the two pegiviruses would be that EqPV1 is a common infection in horses while TDAV (now called EqPV2) is an uncommon infection and neither virus causes hepatitis or any other disease that we know of in horses. Therefore, I would recommend that you not bother testing for these two viruses.

A much more interesting viral discovery was the detection of an equine hepacivirus (EqHV). This RNA virus was originally named non-primate hepacivirus (NPHV) and is the closest known virus to human hepatitis C viruses. It is a common infection of horses throughout the world (in the U.S. 2-7% of adult horses are infected/PVR+ and 30% have antibody to the virus). This virus is hepatotropic and likely causes only a non-clinical hepatitis. EqHV may cause a mild and transient increase in hepatic enzymes and a lymphocytic portal inflammation with more diffuse piece-meal hepatocyte necrosis. The pathology occurs in proximity to both peak viremia and antibody production that typically happens 3-8 weeks after infection. We have not confirmed any EqHV clinical disease in field or experimentally infected horses but this infection can certainly cause transiently elevated liver enzymes in some horses. EqHV is not the cause of the high GGT syndrome in racehorses! Most infected horses appear to clear the virus within 6 months although some are chronically infected. We have not confirmed liver disease in the chronically infected horses but there was a recent report from Europe that made an association between chronic EqHV infection and chronic liver disease and cirrhosis in a horse (Vet Med Sci. 2019 Aug;5(3):372-37). That horse had a mild mixed inflammatory cell infiltrate in the liver on the initial biopsy which supported the diagnosis of a viral infection. Is it possible that chronic EqHV is a cause for chronic hepatitis; such as the "so called chronic active" hepatitis, that has lymphocytic infiltrate in the periportal region? Yes, this could be possible but it is not proven and

Dr. Mason Jager DVM, ACVP is currently researching this possibility; stay tuned. This virus can be transmitted in-utero and by blood inoculation but unfortunately, we do not know other methods of spread (Insects, nasal fecal etc.) on farms.

The most recently discovered equine hepatitis virus is a DNA virus called equine parvovirus-hepatitis (EqPV-H), and this virus has consistently been found in horses with Theiler's disease that occurred 4-12 weeks following administration of tetanus antitoxin or other equine origin blood products. Experimental inoculation of EqPV-H positive TAT into seronegative horses caused a late-onset viremia, seroconversion, and hepatic disease compatible with Theiler's disease. This newly discovered DNA virus is strongly associated with Theiler's disease in the horse; both in equine origin blood product inoculations cases and in farm-outbreak cases where there is no intended blood inoculation. The incidence of Theiler's disease (clinical disease) in previously published outbreaks (Theiler's report in 1918 and several reports from the U.S. in the 1930's when thousands of horses received equine antiserum against Western Encephalitis) reported that approximately 1-2% of inoculated horses developed hepatic failure. This percentage would correspond somewhat with our epidemiologic studies on EqPV-H although we have seen outbreaks (4-5 horses with fulminant hepatitis in association with EqPV-H infection). The latest outbreak that we reported on was on a broodmare farm in Slovenia. Our studies indicate that a high percentage of horses develop some degree of hepatitis following EqPV-H infection and elevations in liver enzymes and liver pathology occurring in association with peak viremia and antibody production that develops several weeks after infection. The disease may be due to an initial immune response to clear the virus from hepatocytes? This virus is hepatotropic and causes hepatocyte necrosis with a predominant lymphocytic/plasmacytic infiltrate and vacuolization of hepatocytes. Most infections are subclinical but a low percentage will have fulminant liver failure with a high mortality. Methods of spread include EqPV-H contaminated blood products with TAT historically being the most common product involved. EqPV-H is resistant to many preservatives offering an explanation as to how it can survive in TAT. Commercial equine plasma products have also been associated with EqPV-H liver failure cases as have heterologous stem cell treatments. Experimental studies indicate that transmission of the virus by this later route is difficult unless EqPV-H positive plasma is added to the stem cell preparation prior to inoculation. EqPV-H infection is often persistent although viremia level generally declines following hepatitis. EqPV-H infection is not believed to be a cause of chronic liver disease! It has been an infrequent case of the high GGT syndrome in racehorses but only in a small and statistically insignificant number of cases. This virus is not transmitted in-utero and other than blood inoculation, we do not know how it is commonly spread on farms. The fact that non-biologic EqPV-H/ Theiler's disease cases were reported only between May and December offers support for insect spread from a positive to a negative horse but this has not been documented. Since the prevalence of EqPV-H infection can be high on certain farms, especially those with previous cases of Theiler's disease, how can we tell if EqPV-H is the cause of liver disease in a virus positive horse? First, if the liver disease is chronic it is highly unlikely that EqPV-H is the cause as chronic disease has not been reported following EqPV-H infection. Second, if the hepatitis is acute and the horse survives there is generally a decline in the serum viremia as the horse recovers; so repeat a qPCR a couple of weeks following recovery from the hepatitis and compare to the initial qPCR taken during the clinical hepatitis. Third, a liver biopsy should have the characteristic findings seen with viral hepatitis as mentioned above.

Horses are more commonly diagnosed with cholangiohepatitis than are most other veterinary related species. Infection is most often a mixed infection of gram-negative enteric rods,

Clostridium spp. and enteric *Streptococcus spp.* Fever, colic, jaundice and in chronic cases weight loss along with very high GGT activity in the serum are characteristic findings. Biliary sludge or stones and dilated bile ducts might be seen on ultrasound exam. These horses rarely present with fulminant encephalopathy. Although the diagnosis is rather straight forward, a biopsy should be encouraged in hopes of identifying the infectious cause. Unfortunately, a positive culture result is reported in only c50% of the cases. Horses with cholangiohepatitis can generally be managed successfully with medical treatments but recurrent cases, cases with severe colic, or cases that have clear evidence of a stone “bulging” at the biliary duct opening in the duodenum require surgical intervention. Horses that have “sludge” rather than well- formed calculi in the bile ducts have a better prognosis for cure with medical treatments. In cases that require surgery, the degree of fibrosis as determined via ultrasound examination and biopsy (if time permits) should be evaluated and prognosis discussed prior to anesthesia and surgery. Confirmation of obstructing biliary stones prior to laparotomy is difficult but a moderate number of cases have a circumferential biliary hyperplasia on microscopic biopsy examination.

Hepatic lipidosis is a common disorder of ponies, miniature horses, donkeys and occasionally a horse with Cushing’s disease. There is usually another predisposing disorder that causes the equine to mobilize triglycerides which can quickly results in hyperlipemia (gross discoloration of plasma with triglycerides > 500mg/dl) and hepatic lipidosis.

CLINICAL SIGNS

The clinical signs of **liver failure** can vary depending most upon duration (acute or chronic), predominant biliary versus hepatocellular injury, and specific causes.

Horses with acute liver failure are more likely to have central nervous system signs as their initial and predominant sign. Horses with chronic liver disease leading to failure commonly (but not always) have weight loss and/or photosensitivity as a clinical finding. Gastric impaction and bilateral laryngeal paralysis are two of many complications that can occur with equine hepatic failure.

The pathophysiology of Hepatic encephalopathy (HE) is complex and likely involves several gut-derived neurotoxins, cerebral and systemic inflammation, cerebral vascular dysfunction, and neuroendocrine abnormalities. High concentrations of blood and cerebrospinal fluid (CSF) ammonia have been most commonly incriminated as causing the pathophysiologic events of HE. Ammonia (NH₃) is thought to pass the blood brain barrier (BBB) by diffusion while the less toxic or non-toxic ionized ammonia (NH₄⁺) is limited to the transcellular route for translocation. Ammonia is believed to play a key role in the development of HE, with glutamate, NMDA (N-methyl-D-aspartate) receptor activity and increased glutamine all playing a central role in ammonia metabolism and HE. Systemic inflammation and bacterial translocation are often part of the syndrome of acute liver failure and HE and treatment for these disorders should be provided. Breakdown products of injured hepatocytes in addition to systemic inflammatory cytokine production increases free radical and metalloproteinase concentrations which can have systemic effects in addition to increasing permeability of the BBB. In addition, serum ferritin and iron (which may approach 100% saturation in horses with liver disease) functions as proinflammatory mediators.

DIAGNOSIS

Biochemical testing is imperative in the diagnosis of both liver disease and liver failure. Biochemical results can be helpful in narrowing the differential diagnosis for the liver failure and, when evaluated over time, can help predict prognosis. Liver specific enzymes include sorbitol dehydrogenase (SDH-cytosol), glutamate dehydrogenase (GLDH-mitochondrial) and gamma glutamyltransferase (GGT) which respectively reflect hepatocellular (SDH, GLDH) and biliary injury (GGT). Aspartate aminotransferase (AST) and alkaline phosphatase (AP) also respectively reflect hepatocellular and biliary injury, but are not liver specific. SDH and GLDH activity can be increased in the serum with even mild hepatocyte injury (endotoxemia, etc.) and these enzymes have a short half-life ($T_{1/2}$) which can be very helpful in determining resolution or progression of the hepatic insult. GGT activity in the serum increases following biliary disease and in the horse, GGT often continues to elevate for a few days (presumably due to biliary hyperplasia) after the hepatic insult is no longer present. HCT and serum iron may be abnormally high in horses with severe liver disease. A small percentage of racehorses may have mild but persistent increases (50-140 IU/L) in GGT without other evidence of liver disease. The elevation in GGT is proposed to occur with oxidative stress associated with maladaptation to training/racing. The only treatment effective in returning the GGT activity to normal is rest. A, presumptive hereditary, hyperbilirubinemia syndrome has been seen; mostly in thoroughbred females.

Liver function tests only become abnormal when approximately 60-70% of liver function is lost and these tests include elevations in direct bilirubin, blood ammonia, prothrombin and partial thromboplastin time, serum iron and gamma globulins (with chronic disease). An increase in direct bilirubin of more than 0.2 mg/dl above normal maximum is a highly sensitive and specific marker of liver failure in equines greater than 3 months of age. When the increase in direct bilirubin is 25% or more of the total bilirubin, this is suggestive of a predominant biliary disease. Septic foals with intestinal ileus sometimes have elevations in direct bilirubin with minimal evidence of hepatocellular dysfunction. This may be due to biliary stasis or resorption of the bilirubin from the intestinal tract. Foals with severe hemolysis may also have marked increases in direct bilirubin. Regardless, treatment should focus on the sepsis and intestinal ileus. There may be a decrease in BUN and albumin (with chronic diseases). Abnormally high serum or plasma bile acids (> 20 $\mu\text{mol/L}$) have been reported to be an early predictor of liver failure and when these remain high they indicate a poor long term prognosis. Concentration of bile acids can be an accurate predictor of prognosis with chronic liver disease and marked hepatic fibrosis but they are not good predictors of prognosis with acute hepatic disease. Some healthy foals may have values above 30 $\mu\text{mol/L}$ until several weeks of age. Serum triglycerides are increased in equines with hepatic lipidosis. In foals with hepatic failure, hypoglycemia is often present, while in adult horses, blood glucose is generally normal or increased. Ammonia may be high in horses with liver failure but this is inconsistent. Other causes of hyperammonemia without liver disease include porto-systemic shunts (ammonia and bile acids are almost always high but serum liver enzymes are usually normal), meconium impaction in foals, a presumed hereditary hyperammonemic syndrome in weanling Morgans, uremic encephalopathy and the rather common enteric hyperammonemia syndrome in adult horses.

Ultrasound examination and liver biopsy are the two most commonly used ancillary tests for detecting liver disease. Ultrasound exam may reveal dilated bile ducts, biliary sludge, biliary stones, hepatic fibrosis, hepatomegaly, smaller than normal liver (very subjective), hepatic lipidosis and hepatic masses. Liver biopsy is best performed after the liver has been visualized on ultrasound exam on either the right or left side. Liver biopsy results are used to determine

amount of fibrosis, inflammation, predominant location of disease and for culture purposes. Although bleeding from the biopsy site may be a concern with liver failure due to the commonly increased PT and PTT, this has not been a clinical problem as platelet counts are generally normal or at least not severely decreased. There is no evidence that prophylactic transfusion of plasma helps to prevent procedure-related bleeding.

TREATMENTS

Treatments of liver failure or liver disease will vary depending on cause. Supportive treatments for most cases of equine liver failure include crystalloid therapy with supplemental dextrose and potassium.

Treatments for hepatic encephalopathy (HE) revolve around decreasing enteric- derived neurotoxins (primarily ammonia), decreasing cerebral edema, correcting glucose, electrolyte and acid-base abnormalities, maintaining perfusion and oxygenation to the brain and other vital organs. Horses with HE can have propulsive cortical signs which may require sedation in order to properly attend the horse and prevent injury to the horse or humans. A low dose of detomidine (5-10 µg/kg IV) may suffice. It is important to not overly sedate a horse with HE as that might cause excessive lowering of the head in the standing horse and promote cerebral edema in addition to the potential negative effects on the brain, liver, and other organ perfusion. If additional seizure or propulsive behavior control is required, phenobarbital administration would be preferred. Diazepam should not be used in horses with HE as it can induce astrocyte swelling and worsen HE. Clinical experiences to support this comes from a foal with PSS that we sedated with diazepam in order to place a jugular catheter and the foal developed seizure immediately following treatment. There is also a report of 3 horses with phosphine toxicity and liver failure that developed neurologic signs soon after receiving diazepam. Ideally, sedatives should be avoided in HE. The next stepwise treatment for HE would be to correct intravascular fluid, electrolytes and glucose abnormalities. A normal or slightly high sodium content fluid with additional potassium chloride (20 mEq/L) added is an acceptable initial crystalloid therapy for HE. Supplemental potassium is generally recommended since the horses with HE are anorexic and would most likely be deficient in total body potassium and hypokalemia is known to increase renal proximal tubular ammoniogenesis with the increased ammonia being returned to circulation and potentially worsening the signs of HE. One of the most important goals in the treatment of HE is to reduce in blood and CSF ammonia concentration. The primary means for reduction of blood/CSF ammonia is to reduce the production or absorption of ammonia from the gut. Therapeutic options include oral lactulose or neomycin (10 mg/kg PO q8h) or another poorly absorbed antibiotic. Metronidazole has equal effect on decreasing intestinal ammonia production but it is well absorbed, metabolized by the liver and may have neurotoxic effects. It may be preferred to combine neomycin with lactulose initially if the ammonia is very high. Neomycin decreases ammonia production via its effect of microbial population and decrease in ammonia producing bacteria. Lactulose, a poorly absorbed carbohydrate, decreases ammonia when bacterial degradation of lactulose in the large bowel results in increased H⁺ production and conversion of some ammonia ions (NH₃) to poorly absorbed NH₄⁺ (ammonium) salts. Orally administered antibiotics should not be prolonged beyond 1-3 days to lower the risk of antibiotic-associated diarrhea. It would be ideal if these treatments softened the stool (a cathartic effect) without causing diarrhea. If the horse is eating feeds with moderate amounts of carbohydrates and high branch chain amino acid should be administered every 2-4 hours. Treatments to decrease neuroinflammation and disruption of the BBB are mostly unproven but include N-acetylcysteine, minocycline, neurosteroids such as progesterone or allopregnanolone and hypothermia. Horses with acute liver failure should receive supportive treatment for systemic

inflammation and possible bacterial translocation as intestinal hyperemia and edema are common findings on postmortem of horses dying with hepatic failure. .

Pentoxifylline is often recommended for inflammatory and/or fibrosing liver diseases. Ursodeoxycholic acid (Ursodiol ;15 mg/kg q 24 PO) should be considered in any horse with liver disease and evidence (laboratory or ultrasonographic) of decreased bile flow. I have now used or recommended this treatment in numerous horses with cholangiohepatitis and most of these horses have recovered. I now consider this a potentially valuable drug in the treatment of biliary diseases in horses. Antibiotics should be administered if septic cholangiohepatitis is a possibility. Intravenous penicillin (or ampicillin), gentamicin and metronidazole are reasonable choices as are the combinations of enrofloxacin and metronidazole, trimethoprim sulfa and metronidazole or chloramphenicol per os. Although hepatocellular function is relatively well preserved in most horses with cholangiohepatitis, it is probably best not to administer metronidazole at the highest recommended dose to prevent the possibility of toxic serum concentrations. Hepatic lipidosis treatments include: treat the predisposing disorder, fluids including dextrose (unless the animal is already hyperglycemic) and potassium, enteral or parenteral nutrition if possible are very important and regular insulin (0.1- 1.0 IU/Kg) (or 0.4 IU/kg q24h Zn insulin) as needed for hyperglycemia.

In horses with chronic progressive liver disease not associated with infection, prevention of fibrosis development or progression is key. The activation of hepatic stellate cells (HSC) in response to liver injury is considered a key cellular event that drives the liver fibrosis. Pentoxifylline (7.5 mg/kg P.O. q12h), milk thistle/silymarin (not sure this helps but clients like to use it because they have read about it on the internet), colchicine (0.03 mg/kg P.O. q24h- this is an excellent treatment to inhibit fibrosis but expensive!), zinc supplementation and steroids (prednisolone 0.5-1.0 mg/kg P.O. q24h)) are all used to inhibit fibrosis. Although S-adenosylmethionine, silymarin, and vitamin E are popular "hepatoprotectants" there is not clear evidence for their value in treating liver disease in the horse. Oral administration of S-adenosylmethionine as an intact capsule or tablet may not be clinically effective in horses. Once the capsule or tablet is dissolved, gastric acidity may inactivate the product. An initial thought may be that this may be less problematic for horses that are eating roughage and maintain a relatively high gastric pH; unfortunately, absorption of the drug is inhibited by feeding. Supplementation with vitamin A should be avoided in horses with chronic liver disease as vitamin A can promote fibrosis.

Prognosis

Prognosis is variable in horses with liver failure depending upon the cause! Theiler's disease horses are usually "either dead or on their road to recovery" within 5 days after onset of clinical signs and beginning treatments. Chronic active hepatitis cases often respond to medical treatments and treatment continued until the GGT is normal. After removing therapy approximately 50% relapse. Cholangiohepatitis cases often have a good prognosis if medical treatment begins to work in 7-10 days, there is not severe fibrosis, and multiple stones are not seen on ultrasound exam. Toxic causes such as pyrrolizidine alkaloid toxicosis and liver failure following multiple blood transfusions for N.I. in foals generally have a poor prognosis. Hepatic lipidosis has a good prognosis if the predisposing disorder is resolved and there is a rapid and dramatic response to medical therapy. Portosystemic shunts have been surgically corrected in approximately 50% of the cases reported.