Liver Disease Primer

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Abstract: Liver disease in horses are difficult to recognize until clinical signs manifest, by which time the prognosis is drastically unfavorable. The following presentation highlights recognition and values of diagnostics performed in patients suspected for liver disease. The presentation will conclude with discussion on two recent cases seen at Auburn University Large Animal Teaching Hospital (AULATH).

Keywords: Liver disease, Theiler's, hemochromatosis

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Introduction

This presentation highlights how to recognize liver disease, discuss values of the diagnostic tools used in suspect cases of liver disease, and present two recent cases seen at Auburn University Large Animal Teaching Hospital (AULATH). Since this cannot be a complete and exhaustive investigation into hepatic diseases and particularly therapeutic modalities, the cited book chapters and other resources will provide more details on this topic.

Liver disease in horses, while not common, can often be hard to diagnose until the prognosis is poor. Similar to renal disease, liver disease often manifests only after most (>70%) of organ function has been compromised[1]. Therefore, early recognition and treatment of liver disease is a challenge. In the case of the liver, this is attributed to the liver's hardiness and profound ability to regenerate[1]. The liver functions in a variety of roles in the body, including energy processing and storage, detoxification and metabolism of various compounds, protein production

(albumin, globulin, coagulation factors, inflammatory proteins, etc.), and more[1,2]. Therefore, when liver function is compromised, dysfunction is reflected in these particular parameters.

Suspecting and Diagnosing Liver Disease

Clinical Signs and Differentials

Clinical signs of liver disease can be non-specific, including lethargy, inappetence and associated weight loss, neurologic deficits, jaundice/icterus, bleeding abnormalities, ventral/ dependent edema, secondary photosensitization, diarrhea, decreased perfusion, and hypoglycemia. Potential causes of hepatic disease include toxic, inflammatory, infectious, toxic, metabolic, genetic, and other causes. These categories and specific differentials, while not comprehensive, are listed below in **Table 1**[1-3]. It is important, however, that other disease processes are ruled out, because of the lack of specificity in the manifested clinical signs. Examples (non-exhaustive) of diseases to exclude include neoplasia, myeloencephalidities (protozoal, viral, bacterial, etc.), hemolysis, colitis, and more.

Table 1: Differentials for Hepatic Disease

Inflammatory & Infectious	Toxic	Genetic	Other
Cholangiohepatitis ± Cholelithiasis Enteritis Tyzzer's Theiler's (suspected viral origin, EqPV-H) Others hepatitis viruses: TDAV NPHV EqPgV	Pyrrolizidine alkaloids Alsike clover Panicum grass Mycotoxins Drugs Iron Copper	Portosystemic shunt Processing defects (ammonia, bilirubin)	Hepatic lipidosis Portal vein thrombosis Right dorsal displacement Neoplasia Foals: Umbilical vein abscess

Existing diagnostics can be differentiated into the following categories listed in **Table 2**. Diagnostics performed in liver disease should investigate and answer the following questions:

- What is the etiologic agent causing liver disease?
- What is the extent of liver damage and prognosis for the patient?
- What is the therapeutic plan (if possible)?

Table 2 Classification of various diagnostics performed with suspect liver disease patients

Evidence of liver damage	Evidence of impaired liver function	Identifying etiologic agents
Liver enzymes (specific and non-specific) Architecture: Ultrasonography Histopathology and cytology (biopsy)	Bile acids Bilirubin Albumin/ globulin Ammonia Others (see below) Architecture Ultrasonography Histopathology and cytology (biopsy)	Bacterial culture and susceptibility (biopsy) Histopathology (biopsy) Molecular testing

Clinicopathologic indicators: assessing liver damage and function

Most serum biochemical panels will have some assessment for liver disease. It is important to note that not all biochemistry machines are equal, and may not have these enzymes readily available to measure. Obtaining blood for serum biochemistry panels in liver disease should focus on determining presence of liver damage, and whether evidence of liver function has been affected[1]. The liver specific enzymes in horses are gamma-glutamyl-transferase (GGT[*4]), sorbitol dehydrogenase (SDH), and lactate dehydrogenase (LDH)[1,2]. Other enzymes exist that can indicate other organs, such as aspartate aminotransferase (AST), and serum alkaline phosphatase (AP)[1,2].

[*] GGT can be found in secretions from other organs such as the mammary gland and renal tubules. However, the primary source of serum/ plasma GGT is from the hepatobiliary ducts, and therefore indicates hepatic pathology.

Abnormalities in these values should be used simply as an indicator of liver disease presence or damage, and not as a means of quantifying deficits in liver function[1]. In contrast, liver function tests highlight some of the liver's key roles, including conjugation, metabolism, and synthesis. These are reflected in concentrations of bilirubin, blood ammonia, bile acids, albumin, globulin, as well as coagulation panels and other parameters (prothrombin and partial thrombin time)[1,2]. At AULATH, a typical "large animal liver panel" consists of GGT, SDH, total bilirubin, bile acids, albumin, and globulin. While these values as a whole help in prognosticating each patient, no case should be determined from a single test or value[5]. For example, 40% of horses with signs of hepatic encephalopathy in one study survived 6 months following discharge[5].

Ultrasonography

Performing an ultrasound examination of the liver is a good non-invasive tool in determining the liver architecture. Remembering the motto "SLK" ("spleen-liver-kidney") ordering from most to least echogenic, echogenicity of the liver can be referenced to the spleen between the 6th to 9th intercostal spaces[6]. Increased echogenicity in the liver were consistent with moderate fibrosis, severe hemochromatosis, and moderate to severe biliary hyperplasia on histopathology, therefore carrying a poorer prognosis[5]. Choleliths can also be observed as a hyperechoic object with an acoustic shadow down-field[6]. Abnormal findings on liver ultrasound warrant further investigation via liver biopsy.

Liver biopsy

Liver biopsy is considered the most sensitive and specific antemortem diagnostic testing in suspected equine and human hepatopathy cases[1,2,7]. It is useful for determining the presence of liver disease, provide a specific diagnosis, guide therapeutic choices and determine prognosis of cases[2,7]. Although in the past, blind percutaneous liver biopsies had been performed on the right

side of the horse between the 9th and 16th intercostal spaces, current recommendations are to perform this procedure with the use of ultrasound machine to identify the ideal liver biopsy site[1,2]. This is to minimize potential complications including abscessation from puncturing the gastrointestinal tract, or lung. It has been stated that in light of the beneficial information obtained by liver biopsy, it should be used more so in cases at an earlier stage (patients not in liver failure)[2].

A coagulation panel is typically performed up to 24-36 hours prior to performing a liver biopsy[1,2,8]. However, even in the face of known abnormalities on the coagulation panel, liver biopsies have been performed with no association between coagulation profile abnormalities and complications were observed[8]. The value of performing a coagulation panel is that it allows the clinician to prepare and if needed, act on potential complications should they arise in the coagulopathic patient. In human and equine medicine, this includes providing fresh frozen plasma[8].

Managing client expectations, therapy, and patient at-home care

Like many diseases, early recognition and response to liver disease carry a better prognosis in horses. Unfortunately, once clinical signs of liver disease manifest and are recognized, prognosis for long term recovery become less favorable with one study indicating 38 times less likely for survival with manifestation of clinical signs[5]. However, recognition of one horse in the herd with liver disease warrants investigation of the remaining herd mates' GGT at minimum[2], and if abnormal, performing a biopsy as potential subclinical cases have better prognosis of recovery. If therapy is possible and pursued, clients should expect a long-term treatment, with re-evaluation within 1-3 months[2]. If there is no improvement at this time, repeat biopsy is recommended[2].

Recent Liver Cases at AULATH

Case 1

An 11-year-old American Quarter Horse gelding presented to AULATH as a referral with clinical signs consisting of lethargy, anorexia, and jaundice. The gelding lived on a dirt and grass pasture with 4 other horses that did not show any abnormalities. There was no known administration of equine biological product.

Upon presentation, the gelding's mentation was quiet, dull, but responsive. His physical and neurologic exams were otherwise unremarkable. Trans-rectal palpation revealed no significant abnormalities. Complete blood count revealed hyperfibrinogenemia (500 mg/dL). Serum biochemistry analysis revealed severely elevated liver enzymes (SDH 82.8 IU/L, AST 1831 IU/L, GGT 56 IU/L, reference ranges are 1.9-5.8 IU/L, 226-366 IU/L, and 7-54 IU/L respectively), hyperglobulinemia (4.6 g/dL, reference range 2.62-4.04 g/dL), hyperbilirubinemia (total 8.7 mg/dL, reference range 1.0-2.0 mg/dL), hyperferremia (311 µg/dL, reference range 73-140 µg/dL), elevated bile acids (114.3 µmol/L, reference range <14 µmol/L), severe hyperammonemia (201 µg/dL, reference range 13-108 µg/dL), and hypomagnesemia (total, 1.3 mg/dL, reference range 1.7-2.1 mg/dL). Abdominal ultrasound examination revealed the liver in a small ultrasound window, with no gross abnormality in echotexture, and increased free peritoneal fluid present. Fluid from abdominocentesis revealed low protein transudate consistent with ascites (yellow, clear, total protein 0.5 g/dL, TNCC 120 cells/µL) . Blood coagulation profile performed prior to ultrasound-guided liver biopsy revealed mild thrombocytopenia (73000 cells/µL, reference range 100,000-600,000 cells/µL), and prolonged PT and APTT times (16.7 and 41.9 seconds respectively, reference ranges 11.2-13.4 seconds and 31-37 seconds respectively). The biopsy was submitted for cytology, bacterial culture, and histopathology. Cytology revealed moderate

anisocytosis, anisokaryosis, and more than expected lymphocytes with some erythroid and myeloid precursors. Culture was pending (ultimately negative for growth). Histopathology results were consistent with chronic hepatitis and degeneration (lymphohistocytic and neutrophilic hepatitis).

The gelding was initially admitted for hospitalization. Therapy consisted of supportive care (IV fluid therapy,), broad spectrum antimicrobial therapy (trimethoprim sulfamethoxazole, 30 mg/kg PO BID, neomycin 4 mg/kg PO q. 12h) and anti-inflammatory therapy (flunixin meglumine, 1.1 mg/kg PO q. 12h). Despite therapy, the gelding deteriorated, displaying neurologic signs including head-pressing and dysphagia. The gelding was euthanized due to poor clinical prognosis, and submitted for necropsy. Gross post-mortem examination revealed a smaller liver (1% body weight, normal is greater than 1.5% body weight[9]) and described as small, flaccid, and atrophic. Histopathology of the liver revealed consistent findings of degeneration. Additionally, presence of Alzheimer type II astrocytosis and satellitosis was present within the cerebrum. PCR was performed on serum and liver samples, which returned positive for equine parvovirus (EqPV-H).

Theiler's Disease Discussion:

Theiler's Disease, also known by the names equine serum hepatitis and idiopathic acute hepatic necrosis, is an acute hepatitis whose etiologic agent is currently unknown, commonly associated with recent administration of biologics such as plasma or tetanus antitoxin (4 to 24 weeks[1,10]). An infectious etiology has been suspected for some time, with potential agents historically including Theiler's Disease associated virus (TDAV, pegivirus D), non-primate hepacivirus (NPHV, hepacivirus A), and equine pegivirus (EPgV, pegivirus E)[9-11]. A recent multi-institutional prospective study performed this year suggested a strong suspicion of equine

parvovirus (EqPV-H), following 18/18 horses enrolled testing positive for EqPV-H[9]. A later study found that 9/10 enrolled horses diagnosed with acute hepatitis with no prior exposure to biologics tested positive for EqPV-H, and more than half of herd mates also tested positive[11]. The current mechanism of transmission is still unknown at this time[9,11].

Case 2

An 11-year-old Pony of the Americas mare presented to AULATH as a referral for 6-week history of lethargy and anorexia. Evaluation by the referring veterinarian revealed elevation in liver enzymes and indications of liver dysfunction.

Upon initial physical examination, the mare was quiet, alert, and responsive. She had a body condition score of 5/9 and weighed 943 pounds (429 kg). No abnormalities were noted on initial physical examination. Abdominal ultrasound examination was suggestive of hepatomegaly and a structure with similar echogenicity to the spleen was appreciated on the right side of the abdomen. No abnormalities were noted on transrectal palpation. Abdominocentesis analysis determined the sample as protein-poor transudate (TP 1.1 g/dL, TNCC 830 cells/µL). Liver panel revealed elevated GGT (638 IU/L, reference range 7-54 IU/L), and elevated bile acids (57.2 µmol/L, reference range <14 µmol/L). Coagulation profile showed mild increase in APTT (40.2 seconds, reference range 31-37 seconds). Ultrasound-guided liver biopsies performed were submitted for cytology and histopathology. Cytology revealed anisocytosis and anisokaryosis of hepatocytes, with pigment staining. Histopathology revealed 80% of Kupffer cells (macrophages) and hepatocytes containing stains positive for Prussian blue (hemosiderin). Moderate amounts of fibrous tissue, bile duct hyperplasia, and moderate numbers of inflammatory cells (plasma cells, macrophages) were also appreciated. Samples were submitted revealing serum iron of 331 µg/dL (reference range 91-240 µg/dL) and total iron binding capacity unable to be quantified (270-500

μg/dL). Serum ferritin was measured to be 4062 ng/mL (reference range 85-726 ng/mL). These are consistent with a diagnosis of hemochromatosis.

Hemochromatosis Discussion

Hemochromatosis (iron toxicosis) is a rare disease that occurs presumptively due to excessive ingestion of dietary iron over a long duration[12,13]. Investigation by Theelen et al. in the Netherlands determined in their study pool of 21 horses and one donkey the shortest exposure of 9 years, and based on high iron content in their drinking water, was suspected to be the source of ingestion[13]. One study could not induce iron toxicosis in ponies administered oral iron over the course of 8 weeks indicating the gradual nature of this condition[14]. A diagnosis of iron toxicosis can be guided by increased serum iron and transferrin saturation greater than 80%, in addition to serum/plasma biochemistry analysis consistent with hepatic damage and dysfunction[13]. Definitive diagnosis is made by histopathologic presence of the liver with diffuse deposition of hemosiderin[12,13]. Current therapy is supportive. In human medicine, therapeutic phlebotomy and chelation therapy have been utilized[13]. It is presumed that although iron toxicosis is fatal, the progression of disease is slow, and horses may survive for several years following diagnosis[13]. However, identification of the high iron source should be made, for the health of other animals as well as owners.

References and Recommended Reading:

1. Divers TJ. The Equine Liver in Health and Disease. American Association of Equine Practitioners (AAEP) Convention 2015;66-103.

2. Durham A. An Up-to-Date Approach to Investigating Equine Liver Disease. *ACVIM Forum*, 2018.

3. Davis JL, Blikslager AT, Catto K, et al. A Retrospective Analysis of Hepatic Dysfunction in Horses with Proximal Enteritis (1984-2002). ACVIM Forum 2003.

4. eClinPath.com CU. GGT, 2013.

5. Durham A, Newton J, Smith K, et al. Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. *Equine veterinary journal* 2003;35:542-547.

6. Reef VB. Adult Abdominal Ultrasonography. *Equine Diagnostic Ultrasound*: Saunders, 1998.

7. Durham A, Smith K, Newton J, et al. Development and application of a scoring system for prognostic evaluation of equine liver biopsies. *Equine veterinary journal* 2003;35:534-540.

8. Johns I, Sweeney R. Coagulation abnormalities and complications after percutaneous liver biopsy in horses. *Journal of veterinary internal medicine* 2008;22:185-189.

9. Tomlinson JE, Kapoor A, Kumar A, et al. Viral testing of 18 consecutive cases of equine serum hepatitis: A prospective study (2014-2018). *Journal of veterinary internal medicine* 2019;33:251-257.

10. Tomlinson JE, Van de Walle GR, Divers TJ. What Do We Know About Hepatitis Viruses in Horses? *Veterinary Clinics: Equine Practice* 2019.

11. Tomlinson JE, Tennant BC, Struzyna A, et al. Viral testing of 10 cases of Theiler's disease and 37 in-contact horses in the absence of equine biologic product administration: A prospective study (2014-2018). *Journal of veterinary internal medicine* 2019;33:258-265.

12. Brown P, Whitbread T, Burden F, et al. Haemosiderin deposition in Donkey (Equus asinus) livers: Comparison of quantitative histochemistry for iron and liver iron content. *Research in veterinary science* 2011;90:284-287.

13. Theelen M, Beukers M, Grinwis G, et al. Chronic iron overload causing haemochromatosis and hepatopathy in 21 horses and one donkey. *Equine veterinary journal* 2019;51:304-309.

14. Pearson EG, Andreasen CB. Effect of oral administration of excessive iron in adult ponies. *Journal of the American Veterinary Medical Association* 2001;218:400-404.