Equine Hepatitis Viruses

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1. Introduction

Since 2011, four viruses have been described in the context of equine hepatitis. Two of these, Equine pegivirus (Pegivirus E) and Theiler’s disease associated virus (Pegivirus D), have since been shown to be nonpathogenic and unrelated to liver disease.1 Equine parvovirus-hepatitis (EqPV-H) has been revealed as the cause of Theiler’s disease and mild acute hepatitis, and Equine hepacivirus (EqHV) has been implicated in cases of mild acute and severe chronic hepatitis.

2. Equine Parvovirus-Hepatitis and Theiler’s Disease

History of Theiler’s Disease

A highly fatal form of acute liver failure occurring weeks after administration of equine antiserum for African Horse Sickness was first described by Sir Arnold Theiler in the early 1900s.2 Horses demonstrated acute or peracute onset of liver failure, with hepatic encephalopathy and sudden death as predominant clinical presentations. The syndrome has been observed periodically since then in many countries. Theiler’s disease most often occurs 4 to 10 weeks after administration of an equine-origin biologic product, such as an antitoxin or plasma. Tetanus antitoxin is the most frequently implicated product, likely because it is the most frequently used equine-origin biologic product.3–10 There are also many cases that occur without any history of equine-origin biologic product administration. Sometimes these are horses on the same farm as a biologic-associated Theiler’s disease case, and sometimes there is no history of biologic product use on the farm.2,11 These nonbiologic cases tend to occur from May through November and are often in small outbreaks that span a few weeks.11

Virus Discovery

The natural history of Theiler’s disease demonstrated that the hepatitis was both transmissible and contagious, and a viral cause was suspected. A novel parvovirus, EqPV-H, was discovered by next-generation sequencing of liver from a horse that died of Theiler’s disease and was first reported in 2018.12 Prospective case series showed that this new virus was detectable by serum or liver PCR in 27 of 28 Theiler’s disease cases.3,11 Eighteen of those cases were associated with administration of an equine-origin biologic product, including tetanus antitoxin (n = 12), equine plasma (n = 3), and allogenic stem cells (n = 3).3 Aliquots of the administered products were available for 9 tetanus antitoxin cases and 1 stem cell case, and all were positive for EqPV-H, confirming the route of infection.3 Ten of the cases had no history of equine biologic product administration, and 9 of these were EqPV-H positive.11 Herd-mates of horses with Theiler’s disease also often showed infection and subclinical hepatitis.11 EqPV-H was subsequently demonstrated to be hepatotropic and to cause acute subclinical to mild clinical hepatitis after experimental inoculation.13

NOTES
Clinical Manifestations

Based on the findings in clinical Theiler's cases, their herd-mates, and experimentally infected horses, it appears that EqPV-H infection causes hepatitis with a spectrum of severity ranging from subclinical to fatal. What causes an individual horse to be more severely affected is a topic of ongoing investigation. Hypotheses include variations in individual immune responses, inoculation dose, and a two-hit model where a second insult, such as hepatotoxic plant ingestion, could enhance viral replication or exacerbate pathology.

Theiler's Disease

Also known as acute hepatic necrosis or equine serum hepatitis, Theiler's disease is infrequent but often fatal. Affected horses develop acute liver failure that clinically manifests as icterus, edema, hepatic encephalopathy, and often rapid progression to death (Table 1). Some horses are simply found dead without preceding signs. Clinical pathology indicates both hepatocellular injury, with high aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GLDH), and biliary damage or cholestasis, indicated by high gamma glutamyl transferase (GGT); Table 2. Theiler's cases typically also show functional deficits, as evidenced by high bile acids. Histopathologic examination of affected horses shows diffuse centrilobular hepatocyte necrosis with variable inflammatory infiltrate, biliary reaction, and vacuolar change in surviving hepatocytes.\(^5,9,10,14\)

Mild or Subclinical Hepatitis

Monitoring of herd-mates in Theiler's disease outbreaks has shown that there are often subclinical or mild hepatitis cases alongside the overt Theiler's disease cases.\(^5,11,15\) Some cases have also been identified as incidental findings on screening bloodwork or as mild clinical cases without more severely affected horses on the farm. Mild cases typically exhibit icterus, inappetence, and quiet or dull demeanor, although any signs on the spectrum between subclinical and fatal disease can be observed. Fever has not been observed in subclinical and mild clinical cases.\(^13\) Clinical pathology in these cases is a less severe version of what is seen in Theiler's disease, with mixed increases in hepatocellular enzymes and induction enzymes (Table 2). Histopathology shows individual hepatocyte necrosis with variable lymphocytic infiltrate. In moderate cases, bile ductular reaction can also be observed.

Diagnosis

In classic Theiler's cases of fulminant hepatic necrosis, a simple serum or liver PCR in combination with history, clinical signs, clinical pathology, and histopathology is generally sufficient to establish the diagnosis. In milder or subclinical cases, the diagnosis is not as straightforward. Experimental infections demonstrate that EqPV-H has a prolonged incubation phase of 5 to 8 (median 6.5) weeks, where the horse is viremic before the onset of hepatitis.\(^13\) Hepatitis coincides with peak viremia and a decline in viral titer; however, after the acute infection and hepatitis resolve, many horses remain persistently low-level serum PCR positive for months to years.\(^12,13,15\) Additionally, the prevalence of viremia in apparently healthy horses is around 13%.\(^12\) Therefore, there are many EqPV-H serum PCR positive horses where the virus is not actively causing hepatitis. Additionally, horses have seroconverted and often reached peak antibody titers by the time hepatitis develops, making paired serology unlikely to aid in diagnosis.\(^13\) This means that accurate diagnosis of EqPV-H as the cause of hepatitis presents similar diagnostic challenges as for equine protozoal myelopathy and Lyme disease, where a single positive serum test does not neces-

### Table 1. Clinical Signs of Hepatitis

<table>
<thead>
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<th>Sign</th>
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<tr>
<td>Icterus</td>
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<tr>
<td>Pigmenturia (from direct bilirubin)</td>
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<tr>
<td>Weight loss</td>
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<tr>
<td>Photosensitization</td>
</tr>
<tr>
<td>Respiratory distress (laryngeal paresis)</td>
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<tr>
<td>Ventral edema</td>
</tr>
<tr>
<td>Colic, gastric impaction</td>
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<tr>
<td>Hepatic encephalopathy:</td>
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<tr>
<td>Yawning</td>
</tr>
<tr>
<td>Playing in water bucket</td>
</tr>
<tr>
<td>Central blindness</td>
</tr>
<tr>
<td>Head pressing</td>
</tr>
<tr>
<td>Ataxia</td>
</tr>
<tr>
<td>Circling</td>
</tr>
<tr>
<td>Dullness</td>
</tr>
<tr>
<td>Seizures</td>
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<tr>
<td>Sudden death</td>
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### Table 2. Clinical Pathology Values Observed in Horses with Equine Parvovirus-Hepatitis Infection, Which Developed Fulminant Hepatic Necrosis, Also Known as Theiler's Disease, Associated with Administration of an Equine-Origin Biologic Product (Biologic), or without Such History (Nonbiologic), and in Horses Experimentally Infected with Equine Parvovirus-Hepatitis, Which Developed Subclinical or Mild Clinical Hepatitis

<table>
<thead>
<tr>
<th></th>
<th>Biologic(^5)</th>
<th>Nonbiologic(^11)</th>
<th>Experimental(^13)</th>
<th>Reference intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>1,187 (770–3,426)</td>
<td>2,925 (1,239–6,177)</td>
<td>542 (310–1,068)</td>
<td>222–489</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>134 (68–314)</td>
<td>116 (57–185)</td>
<td>49 (15–233)</td>
<td>8–33</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>15.1 (9.6–24.3)</td>
<td>20.1 (8.7–21.7)</td>
<td>1.9 (1.4–4.3)</td>
<td>0.5–2.1</td>
</tr>
<tr>
<td>Bile acids (μmol/L)</td>
<td>128 (111–171)</td>
<td>90 (76–176)</td>
<td>11 (6–148)</td>
<td>2–10</td>
</tr>
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</table>

Reference intervals shown are from the New York State Animal Health Diagnostic Center. Tests for the Theiler's Cases were performed at multiple laboratories, and laboratory-specific reference intervals varied. Median and ranges are shown.
sarily indicate the agent is actively causing disease. Approaches to improve diagnostic accuracy could include paired serum PCR to observe declining titers and advanced histopathologic imaging such as in situ hybridization to demonstrate viral infection in affected hepatocytes (Fig. 1), thereby linking the virus to the pathology.

Transmission

EqPV-H is known to be transmitted iatrogenically through equine-origin biologic products. Therefore, care should be taken when using and preparing products such as allogenic stem cells and whole blood transfusions to ensure that the donor animal is EqPV-H serum PCR negative. The United States Department of Agriculture has issued a notice requiring that all commercial equine serum and plasma companies ensure their products are free of EqPV-H. There is no evidence to date of vertical transmission. However, EqPV-H has moderately high population prevalence of 13% in Coggins samples, which can be much higher on individual farms. This high prevalence suggests a natural mode of horizontal transmission. Limited data suggests oral transmission is possible; however, a seasonal distribution in Theiler’s cases limited to the summer and fall suggests insect transmission is more likely. This is an open area of investigation.

Treatment and Prevention

As of now, there is no known treatment for EqPV-H, aside from supportive therapies for liver disease. Antiviral drugs targeting paroviruses have not been developed, and therefore direct antiviral treatment is unlikely to be developed. Also, given the rapid progression of Theiler’s disease, and severity of necrosis already present at the time of diagnosis, it is unlikely that an antiviral therapy would be helpful. Therefore, efforts should focus on prevention. Equine-origin biologic products should be confirmed EqPV-H PCR negative before administration. Otherwise, there is no known strategy to prevent horizontal transmission at this time. Effective vaccines have been developed for other paroviruses, e.g., canine parvovirus, and this could be a possibility in the future.

Summary

EqPV-H is known to be a cause of acute to subacute hepatitis that can range in severity from subclinical to fatal. Although serum can remain PCR positive for many months to years, chronic paroviral hepatitis has not been observed. Serum and liver PCR and in situ hybridization can be used to diagnose infection, but results must be interpreted in light of other findings.

3. Equine Hepacivirus and Acute and Chronic Hepatitis

Virus Discovery

In 2011, a group discovered EqHV in samples from dogs by using unbiased high-throughput sequencing to characterize the viral flora of companion animals. The virus was initially named Nonprimate hepacivirus. Subsequent serologic screening of multiple species identified horses, rather than dogs, as the primary host species, and it has since been renamed Equine hepacivirus and classified as hepacivirus A. EqHV is genetically the closest homolog of human hepatitis C virus (HCV) discovered to date. Multiple experimental and natural infections have demonstrated that EqHV is hepatotropic and pathogenic in horses. EqHV is ubiquitous in horses, with 2% to 35% viremia and 22% to 84% seroprevalence in 7 countries across 6 continents.

Clinical Manifestations

As seen with HCV in people, two outcomes are observed with EqHV infection: horses either clear the virus associated with mild hepatitis, or they become persistently infected and remain viremic for > 6 months. One horse has been documented to remain viremic for at least 12 years without hepatitis. There is some evidence that horses that are infected at < 8 months of age might be more likely to develop persistent infection.

Acute Resolving Infections

Experimental infections result in biochemical and histopathologic evidence of hepatitis (Table 3), but disease is mild, and there have been no clinical signs observed in infected horses. Elevations in functional markers, such as direct bilirubin and bile acids, are rarely observed. Histopathologic findings include normal liver or lymphocytic infiltrate and
scattered individual hepatocyte necrosis. Ductular reaction is typically absent.

Chronic Hepatitis

Persistent infection with HCV in people is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. A form of chronic EqHV-associated hepatitis is less well documented; however, there is increasing evidence that this can occur. Recent case reports have demonstrated persistent EqHV infection associated with chronic hepatitis in two horses. One case presented with weight loss and the other with fever, anorexia, and weight loss. The first horse showed primarily an elevation in hepatocellular enzymes (AST) and survived at least 15 months in stable condition. The other showed more predominant elevation in GGT as well as increased bile acids and was euthanized at 15 months due to declining condition. Histopathology was performed, and the horse had severe micronodular cirrhosis with bile duct hyperplasia and portal inflammatory infiltrate. Further delineating the incidence and clinical findings of EqHV-associated chronic hepatitis cases is a field of ongoing investigation.

Diagnosis

As with EqPV-H, determining whether EqHV is the cause of a particular case of hepatitis presents diagnostic challenges and is an open area of investigation. Because of the high prevalence of EqHV viremia, and because horses can remain viremic for many months to years without hepatitis, a single positive serum PCR cannot determine whether EqHV is the cause of disease. In cases of mild acute hepatitis, clearance of viremia and resolution of the hepatitis within 1 to 4 months would be consistent with EqHV as the cause of disease. In cases of chronic hepatitis, ruling out other causes, such as bacterial infection or exposure to toxins, and demonstrating persistent EqHV viremia for at least 6 months would increase the likelihood that EqHV is the cause of disease. PCR findings should also be assessed in the context of liver histopathology.

Transmission

As with EqPV-H, EqHV can be iatrogenically transmitted through contaminated equine-origin biologic products, such as plasma. EqHV can also be vertically transmitted in utero. However, the high prevalence across many countries indicates an efficient method of horizontal transmission. For the related virus, HCV, mosquito transmission has been suggested, although it is not widely accepted as a major route of transmission. EqHV has not been detected in mosquitoes to date. This is an area of ongoing investigation.

Treatment and Prevention

There is no available treatment or prevention for EqHV at this time. Unlike for paroviruses, there are many effective direct acting antivirals that have been developed for HCV, which might have efficacy for EqHV. At least one, sofosbuvir, is predicted to bind EqHV by computer modeling. However, there will be significant development and cost burdens before these medications become available for horses as they are mostly all high cost and of limited availability, even for people. The best approach to prevent transmission currently is to ensure that equine-origin biologic products are PCR negative before administration; however, there is no regulation by the United States Department of Agriculture for this virus at this time. There is also no known means of stopping horizontal transmission since the route is unknown. Vaccines developed for HCV have had disappointing efficacy; therefore, a successful vaccine for EqHV might not be feasible, and efforts will more likely focus on treatments.

Summary

EqHV is a highly prevalent virus that apparently can cause both mild acute hepatitis and severe chronic hepatitis. The incidence and clinical characteristics of EqHV-associated chronic hepatitis need further study. Serum and liver PCR can be used to diagnose infection, but results must be interpreted in light of other findings.

4. Summary

There is a lot of work ongoing to further delineate the epidemiology, diagnosis, treatment, and prevention of both viruses. Based on current knowledge, EqPV-H and EqHV are causes of hepatitis in horses. Both viruses should be considered as differentials for cases of mild acute to subacute hepatitis with evidence of primarily hepatocellular or mixed hepatocellular and induction enzyme elevations and histopathologic findings of lymphocytic infiltrate and/or individual hepatocyte necrosis, with or without biliary ductular reaction. EqPV-H can also cause severe acute hepatic necrosis, also known as Theiler’s disease. EqHV might be a cause of chronic hepatitis characterized by persistent infection and persistent elevation in hepatocellular or mixed hepatocellular and induction enzyme elevations. Histopathologic findings of fibrosis and lymphocytic infiltrate could be consistent with chronic EqHV hepatitis.
Conflict of Interest

The Author acknowledges Drs. Gerlinde Van De Walle, Thomas J. Divers, Charles M. Rice, Troels K. H. Scheel, Mason Jager, Brad Rosenberg, and Amit Kapoor and many co-authors for their mentorship and collaboration on these equine hepatitis virus studies.

Declaration of Ethics

The Author has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Acknowledgments

The Author has received support from the following: Agriculture and Food Research Initiative Competitive Grants 2016-67015-24765 and 2020-67015-31297 from the USDA National Institute of Food and Agriculture, the Jack Lowe Equine Health Funds/Mollie Wilmot Equine Research Fund, the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award K08AI141767, the Harry M. Zweig Memorial Fund for Equine Research, and Boehringer Ingleheim Vetmedica, Inc. 2016 Advancement in Equine Research Award. The content is solely the responsibility of the Author and does not necessarily represent the official views of the funders, including the National Institutes of Health. The text was not reviewed by any sponsor prior to submission.

Support for Research Performed

The Author has received support from the following: Agriculture and Food Research Initiative Competitive Grants 2016-67015-24765 and 2020-67015-31297 from the USDA National Institute of Food and Agriculture, the Jack Lowe Equine Health Funds/Mollie Wilmot Equine Research Fund, the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award K08AI141767, the Harry M. Zweig Memorial Fund for Equine Research, and Boehringer Ingleheim Vetmedica, Inc. 2016 Advancement in Equine Research Award. The content is solely the responsibility of the Author and does not necessarily represent the official views of the funders, including the National Institutes of Health. The text was not reviewed by any sponsor prior to submission.

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