What Do We Know About Hepatitis Viruses in Horses?

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KEYWORDS

• Theiler disease • Parvovirus • Hepacivirus • Hepatitis • Nonprimate hepacivirus • Theiler disease–associated virus • Equine pegivirus

KEY POINTS

- Equine parvovirus (EqPV-H) is hepatotropic, appears to cause subclinical hepatitis in infected horses, and could be the cause of Theiler disease in horses.
- Nonprimate hepacivirus (NPHV), a.k.a. equine hepacivirus, is hepatotropic, typically causes mild, subclinical hepatitis and is not associated with Theiler disease.
- EqPV-H and NPHV are common infections in horses and have prolonged duration of viremia; therefore, virus detection does not prove disease causation.
- Equine pegivirus is not hepatotropic and has no known pathogenic effects.
- Theiler disease–associated virus, another pegivirus, is not hepatotropic and has no known pathogenic effects.

INTRODUCTION

Although many causes of equine liver disease are well described (**Box 1**), the etiology of the most common cause of acute hepatitis and liver failure in horses has remained elusive for more than a century. This condition has been called Theiler disease, serum hepatitis, or idiopathic acute hepatic necrosis.

Theiler disease was first described in 1918 as a condition of acute hepatic necrosis that occurred among thousands of horses involved in a vaccine trial against African Horse Sickness (AHS). Sir Arnold Theiler developed a protocol to immunize horses by administering live AHS virus concurrently with serum from recovered horses. In the first trial of 1148 horses, it was noted that 2% developed fatal hepatic necrosis, 4 to 24 weeks after vaccination. However a similar percentage of 4 of 160

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Box 1
Abbreviated summary of known causes of hepatitis in horses

- Toxic: eg, pyrrolizidine alkaloids, *Panicum* grasses, aflatoxins
- Metabolic: hepatic lipidosis
- Bacterial: ascending cholangiohepatitis, Tyzzer disease in foals (*Clostridium piliforme*)
- Idiopathic: Chronic active hepatitis, (Theiler disease)
- Neoplastic: lymphoma, hepatocellular carcinoma
- New category: Viral: nonprimate hepacivirus (NPHV), equine parvovirus-hepatitis (EqPV-H)

local horses that had not received the vaccine protocol also developed fatal hepatic necrosis, and therefore, the condition was initially thought to be a possibly a contagious condition unrelated to the vaccine. The vaccine protocol was then applied to 1411 army horses and 1154 privately owned horses. Between these 2 groups, the hepatitis mortality rate was 4% to 18%, and was clearly recognized to be related to the vaccination protocol at that point. Sir Arnold Theiler reported on the liver condition, which consisted of severe centrilobular to massive hepatic necrosis, and it became known as Theiler disease or serum hepatitis.[1] Subsequently, a similar vaccination approach was undertaken in the western United States to stop the spread of western equine encephalitis, and a similar secondary outbreak of liver disease was observed in the region.[2]

Since those 2 initial large-scale outbreaks of Theiler disease, the condition has been reported in many countries across the globe in association with the administration of a wide variety of equine biologic products. Implicated biologic products include tetanus antitoxin,[3–9] botulinum antitoxin,[10] antiserum against *Streptococcus equi*,[4,11] pregnant mare's serum,[4] equine plasma,[1,2,5,9,12] and most recently, allogeneic stem cells.[9] However, it also has been reported in the absence of any history of equine biologic product administration, and in horses that are in contact with serum hepatitis cases.[1,9,13,14] Collectively, these findings suggested that the condition was both infectious and contagious, and a viral etiology was suspected. Despite intensive efforts by many groups, no viral agent could be cultured from affected animals, and it was not until the recent advances in unbiased deep sequencing that candidate etiologic agents have been discovered. Since 2011, there have been 4 viruses identified in samples from horses with hepatitis, with some discrepancies between the initial findings and follow-up investigations into the pathogenicity of these viruses. The viruses are Theiler disease–associated virus (TDAV), equine pegivirus (EPgV), nonprimate hepacivirus (NPHV), and equine parvovirus-hepatitis (EqPV-H). The objective of this review was to summarize the current knowledge for each of them, what remains to be explored, and the likely clinical implications of infection with each virus. They will be discussed in order of clinical relevance.

THE "HEPATITIS VIRUSES" OF HORSES

Equine Parvovirus-Hepatitis

Equine parvovirus-hepatitis discovery

EqPV-H was first reported in 2018 by Divers and colleagues[15] (**Box 2**). The virus was detected by unbiased next-generation sequencing of liver obtained from a fatal case of Theiler disease.

Box 2
Equine parvovirus-hepatitis

- Virus prevalence: estimated 13% (based on serum polymerase chain reaction [PCR]).
- Seroprevalence: estimated 15%.
- Transmission: iatrogenic through biologic products, otherwise unknown. Viral DNA present in nasal secretions and feces at peak viremia, but actual transmission via these routes remains to be determined.
- Disease association in experimental models: subclinical-to-clinical hepatitis with marked elevations in liver enzymes and function tests, including bile acids.
- Disease association in clinical cases: 27 of 28 prospectively collected cases of Theiler disease were found EqPV-H positive. All cases that had received equine-origin biologics were positive.
- Clinical implications: likely a relevant cause of liver disease ranging from subclinical liver enzyme elevations to fulminant liver failure.

Equine parvovirus-hepatitis viral biology

Parvoviruses are single-stranded DNA viruses enclosed in a protein capsid. The viral genome is small and does not encode for proteins required for viral replication. For this reason, parvoviruses are typically thought to either require helper viruses to provide replication machinery (the Dependoviruses; eg, adeno-associated virus), or to require actively dividing cells such that the virus can use the host replication machinery (eg, canine parvovirus).[16] More recently, parvoviruses have been identified that can activate and use the host DNA damage repair machinery to replicate in nondividing cells (eg, human bocavirus-1).[17,18] The highest viral load of EqPV-H is in the liver, suggesting hepatotropism (unpublished, Tomlinson, 2019). Although the liver is capable of regeneration and cellular division, it is typically a quiescent organ with minimal dividing cells. Therefore, the replication capacity of EqPV-H in nondividing cells is a topic of ongoing investigation.

EqPV-H is one of the few known members of the *Copiparvovirus* genus. Other commonly known pathogenic parvoviruses include canine parvovirus 2 and porcine parvovirus 1, which are both protoparvoviruses, and human B19 virus, an erythroparvovirus. Although the primary tropism of human parvovirus B19 is the erythroid stem cells, it also infects the liver and has been rarely associated with acute hepatic necrosis, although this finding remains controversial.[19–23]

The complete range of transmission modes for EqPV-H is unknown. Iatrogenic transmission of EqPV-H through administration of equine-origin blood products has been demonstrated.[15] However, the virus is also transmitted among horses that have not received such treatments.[14] Although other methods of iatrogenic transmission (such as sharing needles, rectal sleeves, stomach tubes, or dental equipment) are possible, there must also be methods of natural horizontal or vertical transmission. Other blood-borne viruses of horses, such as equine infectious anemia, are mechanically transmitted by biting flies, such as horseflies and stable flies.[24,25] Insect transmission studies of EqPV-H have not been reported to date. No parvovirus is known to undergo biological vectoring in insects, and therefore, tick or mosquito transmission appears unlikely. Preliminary unpublished data (unpublished, Tomlinson, 2019) demonstrate moderate nasal and low-level fecal shedding of EqPV-H around the period of peak viremia; therefore, nasal and/or oral transmission routes are suspected, but have yet to be proven. This remains a high-priority area of investigation.

Equine parvovirus-hepatitis epidemiology

Investigation of the epidemiology of EqPV-H is still in its infancy. Screening of 100 equine serum samples sent to the New York State Animal Health Diagnostic Center for routine regulatory testing revealed a prevalence of 13% DNA detection and 15% antibody detection.[15] All DNA-positive horses were also seropositive. Horses screened for inclusion in experimental infection studies had a higher prevalence of 16 (31%) of 51 DNA detection, whereas young racing thoroughbreds tend to have lower prevalence (unpublished, Tomlinson, Divers, 2019); however, the prevalence can vary widely across premises. Among horses on properties where at least 1 horse has developed Theiler disease, up to ~70% can be infected.[14] Risk factors, such as age, breed, use, housing conditions, and management practices, have yet to be identified.

Experimental infections and herd monitoring show that EqPV-H DNA can be detected in the serum and/or liver for months to years following infection. There is a prolonged period of viremia before seroconversion, which precedes the onset of hepatitis.[15] Although viremia levels decline after the episode of acute hepatitis,[15] the virus often persists at a low levels. Therefore, detecting EqPV-H DNA or antibodies in the serum of a horse with hepatitis might not be sufficient to make an association between EqPV-H and disease. A drop in viremia during the course of hepatitis and resolution might be a better indicator that the virus is associated with the acute episode of hepatitis. However, the full spectrum of disease that can be attributed to this virus and the diagnostic utility of specific tests have yet to be determined.

Equine parvovirus-hepatitis disease association

EqPV-H was first identified in the liver of a case of Theiler disease.[15] A case series consisting of horses with Theiler disease associated with equine biologic product treatment, found that 18 of 18 horses were infected with EqPV-H.[9] Similarly, a case series of horses with Theiler disease without any history of receiving equine biologic products found that 9 of 10 horses were infected with EqPV-H.[14] The other 3 purported hepatitis viruses, NPHV, TDAV, and EPgV, were not consistently found in either group.[9,14] Experimental infection using either EqPV-H polymerase chain reaction (PCR)-positive tetanus antitoxin (n = 2)[15] or equine serum (n = 5, unpublished, Tomlinson, 2018) demonstrates repeatable subclinical-to-clinical hepatitis. Clinical pathology findings have shown significant changes in some horses, including peak bile acids of up to 148 μmol/L; however, no experimentally infected horse has shown fulminant Theiler disease to date. Two major limitations of the current dataset are (1) a lack of case-control data and (2) a lack of purified inoculum for infection studies. In particular, the lack of a pure inoculum has resulted in misinterpretation of previous results from infection studies with TDAV (see the following section), as the used inoculum was later found to be EqPV-H positive also. Therefore, creating a pure, clonal, EqPV-H inoculum should be a priority to definitively prove EqPV-H can cause hepatitis and Theiler disease.

Hepacivirus A, Also Known As Nonprimate Hepacivirus or Equine Hepacivirus

Nonprimate hepacivirus discovery

NPHV was first discovered in respiratory samples from dogs with airway disease in 2011[26]; however, subsequent studies failed to identify the virus in other canine populations (**Box 3**).[27] NPHV is the closest genetic relative of hepatitis C virus (HCV) known to date. HCV is notoriously difficult to study because it does not grow readily in cell culture and known animal homologs have been lacking, until recently. Due to

Box 3
Nonprimate hepacivirus

- Virus prevalence: 2% to 7% (based on serum PCR).
- Seroprevalence: ~40%.
- Transmission: iatrogenic through biologic products, vertical, otherwise unknown.
- Disease association in experimental models: subclinical hepatitis with mild elevations in liver enzymes.
- Disease association in clinical cases: 1 report of suspected NPHV–associated hepatitis. Found in only 2 of 28 cases of Theiler disease, and always as a coinfection with EqPV-H.
- Clinical implications: likely a relevant cause of mild liver disease. Capacity to cause clinical hepatitis, liver failure, or chronic diseases, such as cirrhosis, chronic active hepatitis, and neoplasia, is unknown.

its genetic similarity to HCV, species tropism studies for NPHV were undertaken, and horses were identified as the primary host of NPHV in 2012.[27] This engendered excitement that horses could potentially provide a valuable animal model for studying hepacivirus biology. A number of studies have been undertaken to determine the tissue tropism, pathogenicity, and immune responses to NPHV infection in horses.

Nonprimate hepacivirus viral biology

NPHV has also been known as equine hepacivirus[28,29] and is officially classified in the family Flaviviridae, genus *Hepacivirus*, species Hepacivirus A.[30] Flaviviruses are enveloped, single-stranded positive-sense RNA viruses. They are frequently bloodborne.

NPHV is hepatotropic, as demonstrated by transfection studies,[31] tissue PCR,[28] and in situ hybridization.[32] Although NPHV can be transmitted through iatrogenic blood or serum transfer,[28,33] the natural route of transmission is unknown. Other examples of the Flaviviridae, such as West Nile Virus, undergo complex transmission cycles, including biological vectoring by mosquitoes, whereas other hepaciviruses are not known to be biologically vectored. Vertical transmission has been suggested from 1 of 4 NPHV PCR-positive mares examined, in which NPHV RNA was found in umbilical cord blood and postnatal foal serum.[34] Other routes of transmission or viral shedding have not been explored to date.

Infection in adults typically lasts a few months, but rarely beyond 6 months, with only 4 of 18 reported experimental inoculations resulting in chronic infection (including 7 unpublished, Tomlinson, 2019).[28,31,33] One horse has been identified as persistently infected for more than a decade by testing previously stored serum samples.[31] It appears that adult horses more readily clear NPHV infection compared with foals that become infected when younger than 8 months old,[28,31,33,35] which could reflect a role of adaptive immunity in viral clearance. In humans, persistent infection with HCV is a major risk factor for hepatocellular carcinoma. It remains to be seen if persistent infection with NPHV in horses can result in chronic liver disease or cancer.

As is observed with HCV, there is delayed seroconversion after infection with NPHV. Adult horses typically do not develop anti-NPHV antibodies until 3 to 8 weeks after inoculation.[28,33] In addition, seroconversion can precede viral clearance by weeks.[28,33] Challenge inoculations in 2 previously infected horses, at 5 months after

clearing the primary infection, demonstrated a nonsterilizing immunity indicated by a short duration of low-level viremia.[33]

Nonprimate hepacivirus epidemiology

NPHV infection in horses occurs worldwide. The virus has been reported in the United States, Great Britain, China, Japan, and Germany.[32,36–42] The virus is also common: 2% to 18% of adult horses are viremic and 22% to 84% are seropositive.[29,36–38,41,42] Seroprevalence appears to increase with age.[32,38] A history of transportation increased the risk of viremia in horses younger than 8 years old, and decreased the risk in horses older than 8 years, potentially suggesting that transportation increases the chance of NPHV infection earlier in life.[38] Similarly, some of the highest rates of viremia and seroprevalence have been detected in thoroughbred racehorses, although a rigorous test of breed association has not been performed.[32,38,43] In addition, there has been no investigation to date into whether this apparent difference could be attributed to management practices and/or genetic susceptibility. NPHV has been detected in a small number of donkeys, although the clinical relevance was not investigated.[29,36]

Nonprimate hepacivirus disease association

Multiple experimental infections with NPHV have demonstrated only subclinical hepatitis. Hepatitis typically occurs around the time of seroconversion.[28,31,32] Histopathologic changes are subtle and include piecemeal hepatocyte necrosis and subjective mononuclear cell infiltrate.[28,31–33] A consensus sequence molecular clone of the virus was developed and transfected into the liver of a horse. This resulted in NPHV infection and mild liver enzyme elevations: peak glutamate dehydrogenase (GLDH) ~2 times reference interval; peak aspartate aminotransferase 1 time reference interval; sorbitol dehydrogenase (SDH), γ-glutamyl transferase (GGT), and bilirubin increased from baseline but remained within reference interval.[31] The horse had delayed seroconversion (11 weeks after transfection), and a prolonged course of viremia (19 weeks).[31] This experiment demonstrated both hepatotropism and pathogenicity of the virus. During experimental infection studies, liver enzymes usually increase above baseline without rising above the upper limit of the reference interval, making both detection of damage and interpretation of clinical relevance difficult. Horses demonstrating hepatitis where liver enzymes do rise above the reference range typically demonstrate mild elevations and no clinical signs.[28,31] A longitudinal investigation of naturally occurring infections in Germany identified 2 horses with GGT up to threefold above and GLDH up to fourfold above the upper limit of the reference interval; however, horses were not screened for concurrent EqPV-H infection.[32]

One study investigated NPHV infection in a group of horses inoculated with plasma from horses that died of Theiler disease.[28] The horses that died were NPHV positive; however, EqPV-H had not been discovered at that time, and coinfection with EqPV-H was not ruled out. The adult recipient horses developed moderate hepatitis, with peak GGT and SDH 1.5-fold to 5.0-fold above the upper limit of the reference range, but without any clinical signs.[28]

There is a single case report of a horse with chronic hepatitis that was chronically infected with NPHV in Hungary.[40] This case was reported before the discovery of EqPV-H, and it is unknown if EqPV-H could also have played a role.

In people, acute HCV infection is often subclinical and the major health effects are related to chronic infection. There is a definitive need to determine whether NPHV could be associated with chronic liver conditions in horses, such as cirrhosis, chronic active hepatitis, and/or hepatocellular carcinoma.

Pegivirus D, Also Known As Theiler Disease–Associated Virus

Theiler disease–associated virus discovery

TDAV was first identified in a serum sample from a horse with nonfatal serum hepatitis. The serum was deep sequenced for RNA virus discovery, after DNA digestion (**Box 4**). TDAV was identified in the affected horse, as well as in the botulism antitoxin that had been administered to this animal. It was also identified in other horses that had been administered the same antitoxin, but not in other horses on the property or neighboring properties. Only horses that had received the TDAV-positive antitoxin developed hepatitis and were shown to be positive for the virus. In addition, experimental inoculation with the botulism antitoxin resulted in successful transmission of the virus and hepatitis in 2 of 4 ponies.[10]

Theiler disease–associated virus viral biology

TDAV belongs to the family Flaviviridae, genus *Pegivirus*, and has recently been classified officially as Pegivirus D.[30] Pegiviruses are enveloped, single-stranded, positive-sense RNA viruses. Although pegiviruses have been identified in many species, there are no examples of pathogenic viruses in this genus. There is controversial evidence that infection with pegivirus might modulate or attenuate disease due to other lymphotropic viral infections, such as human immunodeficiency virus (HIV) in people.[44,45]

Pegiviruses typically have lymphoid tissue or bone marrow tropism.[46] TDAV also appears to have primarily bone marrow tropism, although intrasplenic transfection with viral RNA was able to elicit infection (unpublished, Tomlinson, 2018). TDAV could not be detected in liver samples from an experimentally TDAV-infected horse (unpublished, Tomlinson, 2018, n = 1).

Iatrogenic transmission through contaminated equine blood products has been demonstrated.[10] No evidence of horizontal transmission from 16 infected horses to 14 uninfected horses was detected over a 1-year period, suggesting horizontal transmission is inefficient at best.[10]

Theiler disease–associated virus epidemiology

TDAV is apparently a rare virus. Aside from the initial report, it has been identified in only 1 additional herd that the authors are aware of (unpublished, Divers, 2013) and rarely in serum samples submitted for viral testing. It has been found in pooled equine serum for cell culture,[47] but has otherwise not been found in epidemiologic surveys in multiple countries and regions.[34,36,41,47,48] In 2 case series of horses with Theiler disease in the Unites States, none of the horses had detectable TDAV.[9,14]

Box 4
Theiler disease–associated virus

- Virus prevalence: less than 1% (based on serum PCR).
- Seroprevalence: unknown.
- Transmission: iatrogenic through biologic products, otherwise unknown.
- Disease association in experimental models: unknown. Experimental infection in 3 horses was confounded by coinfection with EqPV-H.
- Disease association in clinical cases: Not present in any of 28 cases of Theiler disease. Present in a herd outbreak of hepatitis after botulism antitoxin administration; however, horses were coinfected with EqPV-H.
- Clinical implications: unlikely to be a cause of clinical disease.

Theiler disease–associated virus disease association

Although TDAV was suspected to be the cause of hepatitis in the first description of the virus,[10] subsequent studies have failed to identify TDAV in clinical cases.[9,14,15,40] Intrasplenic transfection with TDAV RNA yielded TDAV infection, but no evidence of hepatitis (unpublished, Tomlinson, 2018).

After the discovery of EqPV-H, retrospective testing of samples from the botulism antitoxin–associated hepatitis outbreak revealed that the TDAV-positive antitoxin, horses with hepatitis, and experimentally inoculated ponies were all positive for EqPV-H.[10,15]

Pegivirus E, Also Known As Equine Pegivirus

Equine pegivirus discovery

EPgV was also discovered through deep sequencing of equine serum samples (**Box 5**). In this case, samples were submitted from a group of horses in Alabama, where signs of hepatitis were found in multiple animals.[49]

Equine pegivirus viral biology

EPgV, like TDAV, belongs to the family Flaviviridae, genus *Pegivirus*, and has recently been classified officially as Pegivirus E.[30] Like other pegiviruses,[46] EPgV appears to have lymphoid tropism. Limited tissue analysis by PCR showed that EPgV could be detected in peripheral blood mononuclear cells but not in the liver, spleen, kidney, lymph node, brain, or lung of a single EPgV-infected horse.[28] EPgV has been rarely detected in liver samples from horses in a case series of Theiler disease, even when present in the serum.[9,14]

Iatrogenic transmission through contaminated equine blood products has been demonstrated.[28] Although horizontal transmission has not been demonstrated, the high prevalence of the virus suggests that naturally occurring modes of transmission must be present.

Infection results in a prolonged duration of viremia, which has been documented for at least 17 months.[48]

Equine pegivirus epidemiology

EPgV is a common equine infection worldwide. Approximately 1% to 32% of horses are viremic and up to 66.5% are reported to be seropositive.[36,41,48,49]

Equine pegivirus disease association

Although the source material that led to the discovery of EPgV came from a farm with a high prevalence of hepatitis, there was no statistical association between hepatitis and pegivirus infection.[49] Additional epidemiologic surveys and case series of Theiler disease also have failed to find evidence of a link between EPgV and liver disease.[9,14,48]

Box 5
Equine pegivirus

- Virus prevalence: 1% to 32% (based on serum PCR).
- Seroprevalence: 66%.
- Transmission: iatrogenic through biologic products, otherwise unknown.
- Disease association in experimental models: unknown.
- Disease association in clinical cases: not associated with hepatitis.
- Clinical implications: unlikely to be a cause of clinical disease.

SUMMARY

Clinical Relevance of the Hepatitis Viruses

The story of the emerging "equine hepatitis viruses" is an excellent example of the need for rigorous study and characterization of novel infectious agents. Next-generation sequencing has allowed researchers to identify new viruses that have not been amenable to detection by cell culture methods. This presents the problem that it can be exceedingly difficult to generate a well-characterized, pure inoculum, free of other infectious or toxic agents. In that case, studies rely on the use of samples from clinically affected horses, which can contain additional as-yet-unidentified pathogenic factors. This has occurred in the initial description of TDAV and likely in some of the NPHV studies.[10,15,28] This also emphasizes the importance of follow-up studies to confirm the hypothesized pathogenic effect through case series, as was used to disprove the hypothesis that TDAV caused Theiler disease,[9,14,15] and/or by using alternative inocula, such as transfection with viral genomes, as was done for NPHV.[31] In this light, and although there is strong associative evidence that EqPV-H could be the cause of Theiler disease, additional studies are warranted to further prove the association.

Despite all 4 viruses being described in the context of liver disease, only 2 have turned out to demonstrate repeatable liver pathogenicity. Both NPHV and EqPV-H experimental infections generate consistent evidence of mild-to-moderate hepatitis, although it has to be noted that only small numbers of horses have been infected to date. Consequently, the full range of disease attributable to each virus is yet to be explored. Theiler disease is a rare consequence of equine biologic product administration; therefore, we suspect that individual variation in the immune response may be required to instigate fulminant disease. The risk factors associated with acute liver failure are unknown, just as the consequences of chronic infection are unknown.

Once the pathogenic potential of NPHV and EqPV-H are fully delineated, clinicians will likely still face obstacles in establishing effective diagnostic tests for "viral hepatitis." Because both viruses tend toward prolonged periods of viremia, and horses can remain viremic after seroconversion, a single positive serum PCR and/or serology might not be the most appropriate test to determine whether the episode of hepatitis is related to that particular viral infection. A similar diagnostic problem is, therefore, predicted as what faces us in the context of equine protozoal myeloencephalitis and Lyme disease. Additional tests are likely needed and might include immunoglobulin M serology or serial sampling to document decline in viremia. All this is an area for future research.

To the best of our current understanding, EPgV and TDAV are not hepatotropic and of no direct clinical significance, particularly in the area of liver disease. This is similar to what we know about pegiviruses that infect other species. Additional studies with these viruses should be performed to determine if pegiviruses may interact with the immune system in either a beneficial or detrimental manner, similar to what has been suggested to occur during coinfections with human pegivirus and HIV.[44,45]

Are These Emerging or Expanding Viruses?

Due to the recent discovery of these viruses, their historical prevalence and genetic evolution have only been minimally explored to date. Retrospective review of some herd samples have identified the presence of NPHV as long as 12 years ago[31]; however, older samples have not been screened to the authors' knowledge. Likewise, retrospective testing of serum samples from possible Theiler disease cases have identified EqPV-H in samples from as far back as 1981 (unpublished, Tennant, 2013); so, it

is tempting to speculate that these viruses have been circulating in equine populations for decades, at a minimum, and are not truly emerging threats. NPHV appears to be well established in horses across all countries in which NPHV prevalence has been evaluated, and therefore, a significant spread or expansion of the virus seems unlikely. In contrast, the worldwide prevalence of EqPV-H is much less known, due to more recent discovery of this pathogen. It is notable that most Theiler disease reports arise from the United States. If EqPV-H is indeed the cause of Theiler disease, this could indicate a limited geographic distribution of the virus, and a potential that its range could expand through horse movements, shipment of virus-contaminated biologics, or changes in vector ranges, depending on its mechanism of transmission. All this should be investigated in future studies.

Future Directions

Many of the most pressing topics for future studies on these viruses have been summarized throughout this review; however, we would like to propose a few priorities:

- It is essential to confirm the pathogenicity of EqPV-H through additional rigorous study and preferably through experimental infections with purified viral inoculum.
- We need to explore the range of liver diseases than can be caused by NPHV and EqPV-H, and the risk factors that precipitate liver failure versus subclinical disease.
- It will be important to delineate modes of transmission to establish control measures for each virus.
- It would be beneficial to provide more definitive evidence that EPgV and TDAV are not equine pathogens.

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