

STANDARD ARTICLE

Viral testing of 18 consecutive cases of equine serum hepatitis: A prospective study (2014-2018)

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Background: Three flaviviruses (equine pegivirus [EPgV]; Theiler's disease-associated virus [TDAV]; non-primate hepacivirus [NPHV]) and equine parvovirus (EqPV-H) are present in equine blood products; the TDAV, NPHV, and EqPV-H have been suggested as potential causes of serum hepatitis.

Objective: To determine the prevalence of these viruses in horses with equine serum hepatitis.

Animals: Eighteen horses diagnosed with serum hepatitis, enrolled from US referral hospitals.

Methods: In the prospective case study, liver, serum, or both samples were tested for EPgV, TDAV, NPHV, and EqPV-H by PCR.

Abbreviations: ACVIM, American College of Veterinary Internal Medicine; EqPV-H, equine parvovirus-hepatitis; EPgV, equine pegivirus; NPHV, non-primate hepacivirus; TAT, tetanus antitoxin; TDAV, Theiler's disease-associated virus.

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Results: Both liver tissue and serum were tested for 6 cases, serum only for 8 cases, and liver only for 4 cases. Twelve horses received tetanus antitoxin (TAT) 4-12.7 weeks (median = 8 weeks), 3 horses received commercial equine plasma 6-8.6 weeks, and 3 horses received allogenic stem cells 6.4-7.6 weeks before the onset of hepatic failure. All samples were TDAV negative. Two of 14 serum samples were NPHV-positive. Six of 14 serum samples were EPgV-positive. All liver samples were NPHV-negative and EPgV-negative. EqPV-H was detected in the serum (N = 8), liver (N = 4), or both samples (N = 6) of all 18 cases. The TAT of the same lot number was available for virologic testing in 10 of 12 TAT-associated cases, and all 10 samples were EqPV-H positive.

Conclusions and Clinical Importance: We demonstrated EqPV-H in 18 consecutive cases of serum hepatitis. EPgV, TDAV, and NPHV were not consistently present. This information should encourage blood product manufacturers to test for EqPV-H and eliminate EqPV-H-infected horses from their donor herds.

KEYWORDS

horse blood origin biologics, liver failure, parvovirus, tetanus antitoxin, Theiler's disease

1 | INTRODUCTION

Equine serum hepatitis, also known as Theiler's disease or idiopathic acute hepatitis, is a serious and often life-threatening disease of adult horses that was 1st described in 1918 in South Africa by Sir Arnold Theiler.¹ The 1st cases of serum hepatitis in horses reported in the United States occurred during the pandemic of western equine encephalomyelitis in the 1930s.²⁻⁵ The incidence of fulminant hepatitis among horses receiving antiserum in these outbreaks was between 1.4% and 18%.^{1,4} Serum hepatitis has since been described in horses worldwide after treatment with a variety of equine serum products, including tetanus antitoxin (TAT),⁶⁻¹² botulinum antitoxin,¹³ *Streptococcus equi* antiserum,^{7,14} pregnant mare's serum,⁷ and equine plasma.¹⁵ Of these equine biologic products, the disease has been most commonly associated with TAT, possibly because this is the most frequently administered equine blood origin biologic product.⁶⁻¹² The incubation period for clinical disease usually ranges between 4 and 10 weeks after administration of an equine origin biologic, although it can be as long as 14 weeks.^{1,4,8} In the majority of cases, the association of equine serum hepatitis with the parenteral injection of antiserum or plasma suggests an infectious blood-borne cause, and the history, incubation period, and histopathologic findings appear most similar to serum hepatitis (hepatitis B) in human beings.¹⁴

Recently, 3 novel flaviviruses were identified in horses, of which 2, non-primate hepacivirus (NPHV) and Theiler's disease-associated virus (TDAV) were proposed to be associated with liver disease.^{13,16,17}

NPHV, also called equine hepacivirus, is a member of the genus *Hepacivirus*,^{16,18} and its hepatotropism and production of hepatic disease after experimental and natural infection in horses is well documented.¹⁹⁻²⁵ TDAV is a member of the genus *Pegivirus* and was identified during an outbreak of acute clinical hepatitis in horses, 6 weeks after prophylactic administration of botulinum antitoxin of equine origin.¹³ TDAV's close relative, equine pegivirus (EPgV), commonly infects horses in the United States, Western Europe, and China, although this virus is not hepatotropic and has not been associated with hepatic disease.^{17,26,27} NPHV and EPgV are frequently detected in commercial horse serum pools, whereas TDAV is not.^{28,29}

More recently, a novel equine parvovirus (equine parvovirus-hepatitis, EqPV-H) was discovered in the liver and serum of a horse that died of Theiler's disease.³⁰ EqPV-H nucleic acids were also found in the TAT administered to this horse 9 weeks before onset of hepatitis. Experimental administration of EqPV-H-positive TAT samples to 2 horses resulted in EqPV-H viremia 6.4 weeks later, followed by marked biochemical evidence of liver disease in both horses and clinical disease in one of the horses.³⁰

After the discovery of these viruses, a prospective study on field cases of Theiler's disease involving American College of Veterinary Internal Medicine (ACVIM) Diplomates was initiated to investigate the association of these viral infections with naturally occurring cases of serum hepatitis. This report details case information and virus testing of 18 consecutive cases of serum hepatitis.

2 | MATERIALS AND METHODS

2.1 | Prospective clinical case study

In collaboration with North American academic and private referral equine hospitals, we initiated a prospective clinical case study to assess the possible role of the newly identified viruses in the etiology of acute serum hepatitis via a letter sent to ACVIM Large Animal Diplomates at teaching and large referral hospitals. Case definition included (1) acute onset of clinical signs of hepatic failure with laboratory or liver histopathologic findings characteristic of serum hepatitis (Theiler's disease)^{6-8,11,12,14} and (2) a history of receiving an equine biologic product 4-14 weeks earlier. For each case, a diagnosis of Theiler's disease or serum hepatitis was made at the referral practice before submitting samples to the New York State Animal Health Diagnostic Center for viral testing. Cases were enrolled between January 2014 and February 2018, and all submitted (consecutive) cases were included in the study.

2.2 | Sample collection for prospective study

Serum samples collected from horses in the prospective clinical case study were frozen or kept on ice before being shipped from the clinic

TABLE 1 qRT-PCR primer-probe pairs used to detect hepatitis-associated viruses in equine liver or serum samples

Name	Specificity	Sequence	Source
TDAV-UTR171F	TDAV forward	AGGGTTCTTCGGGTAATCC	Chandriani et al ¹³
TDAV-UTR336Rd	TDAV reverse	CCCTCGGACTGAATTrTAGGC	Modified from Chandriani et al ¹³
TDAV-UTR274	TDAV probe	ACCTCCCTACGAAAGGTCGCCAC	This study
QANTI-5UF1	NPHV forward	GAGGGAGCTGRAATTCGTGAA	Burbelo et al ¹⁶
QANTI-5UR1	NPHV reverse	GCAAGCATCTATCAGACCGT	Burbelo et al ¹⁶
NPHV-UTR288	NPHV probe	CCACGAAGGAAGGCGGGGGC	Burbelo et al ¹⁶
EPgV-80F	EPgV forward1	ACCGAGCCGCCCTGTAG	This study
EPgV-163R	EPgV reverse1	CCTGCCACCGCGATCA	This study
EPgV-UTR122	EPgV probe1	TCCTGGCACTGGCCCGAAGC	This study
EPgV-127F	EPgV forward2	GCACTGGCCCGAAGCAT	This study
EPgV-210R	EPgV reverse2	CTGCCCTAACACAATCACAACAC	This study
EPgV-UTR165	EPgV probe2	TTCTTCGGGTAAATCCCGGCCG	This study
EqPV-3218F	EqPV-H forward	ATGCAGATGCTTCCGACC	This study
EqPV-3386R	EqPV-H reverse	GCCCCAGAAACATATGGAAA	This study
EPV-3310	EqPV-H probe	ACCGTAGCGGATTCGGGATCTGC	This study

Abbreviations: EPgV, equine pegivirus; EqPV-H, equine parvovirus-hepatitis; NPHV, non-primate hepacivirus; TDAV, Theiler's disease-associated virus; qRT-PCR, real-time PCR.

of origin to Cornell University for viral diagnostic testing. Liver samples were shipped either frozen or in formalin-fixed blocks for viral testing. When available, equine blood products or a sample of the biologic with the same lot number administered to the horses before the onset of liver failure were shipped on ice for virologic testing. In all cases, prospective sampling and analysis were approved in full by the Cornell University Institutional Animal Care and Use Committee IACUC #2012-0154.

2.3 | Polymerase chain reaction

Viral nucleic acids were extracted from serum or liver with Qiagen Viral RNA Mini kit (catalog no. 52906) according to the manufacturer's instructions. No DNAase treatment was applied. All PCR mixtures used the Path ID multiplex RT-qPCR kit (catalog no. 4442137; Thermo Fisher Sci, Waltham, MA, USA) and 4 μ L of extracted nucleic acids in a 25 μ L reaction volume. The primers are listed in Table 1. Two primer pairs were used for EPgV; a positive result in either pair was considered positive. Primers were used at 0.4 μ M concentration and probes at 0.12 μ M. All PCR reactions were run on the ABI Step-One-Plus Real-Time System and analyzed with StepOne software (Thermo Fischer Sci, Waltham, MA, USA). Real-time PCR conditions included an initial incubation at 48°C for 10 minutes, then 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. PCR methods were validated using the AAVLD accreditation guidelines (data not shown).

2.4 | Statistics

Descriptive statistics of demographic data are provided. Continuous variables are reported as median and range because of non-Gaussian distribution of some variables, as assessed by the examination of skewness, kurtosis, and Q-Q plots.

3 | RESULTS

3.1 | Signalment and biologic product history

Eighteen cases of serum hepatitis were enrolled between December 2014 and February 2018. Demographic data and virologic testing are summarized in Table 2, and greater individual case details are included in Supporting Information Supplemental Table 1. Multiple breeds of horses were affected. There were 6 mares, 1 stallion, and 11 geldings. The ages ranged from 2 to 18 (median 12) years. Of the 18 cases, 12 horses received commercial TAT 4-13 weeks (median 8 weeks) before acute onset of signs of liver failure. The antitoxin (same vial or lot number) was available for testing in 11 cases. In 4 cases, the TAT lot number was narrowed to 2 possibilities and both were tested (Supporting Information Supplemental Table 1). In 2 of these cases, PCR results were the same for both lots. In the other 2 cases, there was a discrepancy between the 2 lots in either NPHV (1 case) or EqPV-H (1 case) status. Therefore, we are confident that EqPV-H-positive TAT was administered to at least 10 of the 12 cases. Of the remaining 6 horses, 3 had received allogenic stem cells as a treatment for soft tissue orthopedic injuries 6.4, 6.7, and 7.6 weeks earlier, and 3 horses received equine plasma 6, 6.4, and 8.6 weeks earlier as colloid treatment, after abdominal surgery in 1 horse and for diarrhea in 2 other horses. Stem cell inoculum (frozen) was available for virus testing from 1 case (case 17) only, and the inoculum was EqPV-H positive. Sera from the donor horses of the remaining 2 cases were tested 20 weeks after inoculation (case 8) and 15 weeks before inoculation (case 12), and both donor horses were PCR negative for EqPV-H. Samples from the commercial plasma given to the other 3 horses were not available for virus (PCR) testing. This commercial plasma was from 2 separate vendors, although 2 cases (cases 13 and 14) received plasma with an identical lot number.

TABLE 2 Demographic data and virologic testing results for 18 cases with equine biologic-product associated serum hepatitis

Biologic administered (number of horses)	TAT (12)	Plasma (3)	Allogenic stem cells (3)
Age (y) (median [range])	11 (2-17)	15 (12-16)	13 (9-18)
Breed	AQH, 6; WB, 2; others, 4	WB, 2; UNK, 1	AQH, TB, WB
Sex	Mare, 6; Stallion, 1; Gelding, 5	Gelding, 3	Gelding, 3
Incubation period (wk) (median [range])	8 (4-13)	7 (6-8)	6 (5-8)
Survival	4/12	2/3	0/3
Serum qRT-PCR*			
EqPV-H	9/9	2/2	3/3
NPHV	2/9	0/2	0/3
TDAV	0/9	0/2	0/3
EPgV	2/9	2/2	2/3
Liver qRT-PCR*			
EqPV-H	6/6	2/2	2/2
NPHV	0/6	0/2	0/2
TDAV	0/6	0/2	0/2
EPgV	0/6	0/2	0/2
Biologic product qRT-PCR*			
EqPV-H	9/9	NA	1/1
NPHV	7/9	NA	0/1
TDAV	0/9	NA	0/1
EPgV	9/9	NA	0/1

Virology testing (in rows indicated by *) is shown as the number of positive samples out of the number of samples tested. Biologic products tested were mainly aliquots of the same lot administered to the actual cases. Four horses had the TAT lot narrowed to 1 of 2 lots; 2 sets had identical virology results and are included in this table; 2 sets had discrepant results reported in Supporting Information Supplemental Table 1 and are not included in this table. Abbreviations: AQH, American Quarter Horse; EPgV, equine pegivirus; EqPV-H, equine parvovirus-hepatitis; NA, not available; NPHV, non-primate hepacivirus; qRT-pCR, real-time PCR; TAT, tetanus antitoxin; TB, Thoroughbred; TDAV, Theiler's disease-associated virus; WB, Warmblood; UNK, unknown.

3.2 | Virology

All 18 horses were positive for EqPV-H infection (Table 2). Serum and liver samples were available for testing in 6 of the 18 cases; serum only was available in 8 cases and liver only in 4 cases; all these samples were positive for EqPV-H. Of the 14 serum samples, 5 were also positive for EPgV, 2 were positive for NPHV, but none of the 14 samples were positive for TDAV. All 10 liver samples were only positive for EqPV-H. Twelve of the 18 horses died ($n = 6$) or were euthanized ($n = 6$) because of the severity of liver failure. One of the horses (case 7) that survived acute fulminant hepatitis was tested 13 months later and still had detectable EqPV-H viremia without any biochemical evidence of hepatic disease.

3.3 | In-contact horses

The newborn foal of case 4 received the same TAT as her dam at foaling. Serum from the foal was also EqPV-H positive (viremia was

100-fold higher than the dam) when tested at 8 weeks of age; the foal was clinically normal; however, no biochemical analysis was performed. After case 5 recovered and returned to the farm, an in-contact horse developed clinical and biochemical findings of liver failure 6 weeks later. This horse was also sent to the university (Missouri) referral hospital, diagnosed with acute hepatic failure, successfully treated, and returned to the farm 1 week later. The serum of this in-contact horse also tested positive for EqPV-H but no biologic product had been administered to this horse, suggesting the possibility of horse-to-horse transmission (from case 5) as has sporadically been observed in serum hepatitis outbreaks.^{1,11} In case 6, the field veterinarian reported that 2 horses had been inoculated with the same lot of TAT after castration and both developed signs of liver failure, although only 1 of the horses (case 6) was referred for hospitalization and included in our study. Case 6 died, although the other horse recovered on the farm and a blood sample from that horse was positive for EqPV-H.

3.4 | Clinical data

Although not a primary aim of the study, information on clinical signs, biochemical findings, and necropsy findings was available for many of the cases and are reported here for clinical interest. The clinical findings in horses that necessitated the initial veterinary examination were reported to be acute onset of neurologic signs in 12 of 16 cases where the initial clinical findings were available. Ten of the 12 horses were reported as having predominantly cerebral signs, including blindness, head pressing, and obtundation, whereas 2 horses had acute ataxia that preceded the cortical signs. Other initial clinical findings noted in the case records that were provided included icterus ($n = 9$), discolored urine ($n = 5$), colic signs with gastric reflux ($n = 2$), and recumbency in 1 horse that was severely hypoglycemic. Pyrexia was only reported in 2 cases. In 7 cases, the owners reported decreased appetite and dullness for 1-2 days before the onset of neurologic signs. Serum biochemistry findings at referral admission are summarized in Table 3. Median percentage direct to total bilirubin was 17% (13%-27%, $n = 7$). Glucose values were reported for 9 horses. Two values were moderately low (60 and 52 mg/dL), and 2 values were severely low (<20 mg/dL) in recumbent horses. Duration of clinical signs before death or euthanasia (median 3 days, range 1-7 days) was available in all 12 horses that died. Information regarding time to clinical improvement after hospitalization was available for 4 of 5 surviving horses, and number of days after hospitalization to clinical improvement was 3, 3, 4, and 7 days. One horse (case 16) was being treated for a chronic respiratory disease and had a complete blood chemistry panel (which was normal) 8 days before onset of liver failure. This was the only horse in the study receiving medical treatment for another condition at the time of onset of liver failure.

Gross findings of the liver were noted on 7 of the necropsy reports, and in all but 1, the liver was reported to be small (normal $>1.5\%$ of body weight³¹) and friable. A reticular pattern was noted in 3 cases. Reports on the microscopic findings in the liver were available in 15 cases that had either biopsy (4 cases) or necropsy reports (11) submitted; 3 horses that survived did not have liver biopsies performed because only a small amount of liver could be visualized on

TABLE 3 Clinical pathology of 18 Theiler's disease cases

	Number of cases	Median (range)	Reference range
AST (U/L)	12	2097 (706-4078)	222-489
GGT (U/L)	13	129 (68-314)	8-33
Total bilirubin (mg/dL)	13	13.4 (7.6-24.3)	0.5-2.1
Direct bilirubin (mg/dL)	7	1.9 (1.3-5.8)	0.1-0.3
Ammonia (mmol/L)	6	249.5 (30.6-692)	Not determined
Bile acids (μmol/L)	6	118.5 (98.7-171)	2-10
Glucose (mg/dL)	9	87 (19-121)	71-113
Hematocrit (%)	13	48 (36-58)	34-46

Data are from the 1st serum biochemistry performed on each horse at hospital admission. Reference range provided is a general range from the New York State Animal Health Diagnostic Center. Tests were run at multiple laboratories and laboratory-specific reference ranges varied. Abbreviations: AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

ultrasonographic examination. Microscopic findings reported in affected horses consistently included acute centrilobular to massive necrosis, collapse of the lobular architecture, and replacement with cellular debris and sometimes hemorrhage. Lesser affected periportal hepatocytes were often described as degenerate, swollen, and containing cytoplasmic vacuoles. In all but 1 case, a mild to moderate lymphocytic/plasmacytic periportal infiltration was noted. Bile stasis and biliary proliferation were noted less commonly. Alzheimer type 2 cells in the brain, consistent with hyperammonemia, were present in 4 of 5 reports that included microscopic examination of the brain. All necropsy and biopsy reports summarized the histologic features as being most suggestive of, presumptive for, or compatible with serum hepatitis.^{1,8,11,12}

4 | DISCUSSION

The 18 cases in our study were (1) clinically and clinicopathologically consistent with previous descriptions of serum hepatitis in horses, (2) all infected with EqPV-H, and (3) rarely infected with the equine flaviviruses that have recently been suspected of causing the disease.^{13,19} When samples of the biologic product or their same lot number were available for virus testing, EqPV-H was found in the products administered to the horses before onset of hepatitis. Despite the limitation of a lack of controls, the 100% EqPV-H prevalence among these 18 cases compared to the low prevalence of 13% EqPV-H viremia among normal horses³⁰ is highly suggestive that this association is significant. These findings are indicative that EqPV-H can be transmitted by administration of equine biologic products and is the likely cause of equine serum hepatitis.

The clinical and histopathologic findings in these cases, along with the knowledge of administration of an equine origin blood product 4-12 weeks earlier, were considered diagnostic for serum hepatitis;^{6-9,11,12,15} therefore, additional testing (eg, heavy metals and other hepatotoxins) was limited. All except 1 horse in our study were in the "typical" 4- to 10-week incubation period for serum hepatitis,^{8,10-12,15} and the longest incubation period was 12.7 weeks. One of 2 adult horses inoculated with TAT containing EqPV-H had clinical signs of liver failure and abnormal liver function test results 12.7 weeks after inoculation, supporting the possibility that some cases of serum hepatitis can develop outside the normal 4- to

10-week incubation period.³⁰ Some potential explanations for differences in incubation time for disease after administration of virus-laden blood products could include (1) different viral loads or specific antibody titers in the biologic products, (2) individual horse differences in the immune responses to infection, (3) partial protective immunity from previous exposure, or (4) concurrent liver injury of another etiology.

Although the overall incidence of clinically recognized serum hepatitis in adult horses receiving TAT is low, TAT has been the most common blood product associated with the disease in the United States for the past 50 years.^{6,7,9,11,12} Our findings concur with those reports as 12 of the 18 cases with serum hepatitis received TAT. Serum hepatitis could be more commonly associated with TAT administration than with administration of equine plasma because of the more frequent administration of TAT to horses or because TAT is produced as a pooled donor product. The latter could increase the risk of virus contamination of TAT compared to plasma, whole-blood products, and allogenic stem cell inoculations, which are more commonly single donor products. Commercial TAT is usually heat treated (60°C for 1 hour) for the purpose of virus inactivation, and both phenol and thimerosal are added as preservatives. If effective in sterilizing the product, such treatments could leave detectable viral nucleic acids in TAT that are no longer infectious. However, although this form of heat treatment of blood products is known to inactivate heat-labile viruses such as lentiviruses,³² the parvoviruses (and especially animal parvoviruses) are resistant to both heat inactivation and solvent detergent treatments.³²⁻³⁵ Indeed, EqPV-H can be successfully transmitted using heat-treated, commercially available TAT.³⁰ In contrast to EqPV-H, it appears that transmission of the flaviviruses NPHV and EPgV might have been effectively reduced or eliminated by heat and chemical treatment of equine TAT used for the cases in the present study. This is supported by the fact that among 9 cases that had serum and the administered lot number of TAT tested, TAT was positive for EPgV in all 9 lot inocula and NPHV was positive in 6 horses, but only 2 horses were positive for EPgV and 1 positive for NPHV in serum samples. Those flavivirus-positive cases might have been infected either by receiving contaminated TAT that was not properly heat-inactivated or by exposure to these viruses via another source before antitoxin administration. Because the virus prevalence rate of both NPHV and EPgV in the adult horse population is approximately 15%, the latter is a plausible explanation.^{26-28,36}

The association of equine plasma administration and serum hepatitis is also well documented. The time between plasma administration and development of hepatic failure in the 3 cases in our study is typical of previous plasma-associated cases of serum hepatitis.^{13,15} The 2 plasma products (2 horses received the same lot number) administered to these horses were not available for virus testing; therefore, the spread of infection by commercial plasma in these 3 plasma-related cases remains presumed.

An association between the allogenic stem cell treatment and serum hepatitis, as occurred in 3 horses in our study, has not been previously reported. The incubation period between the stem cell inoculation and the onset of disease in these 3 horses was typical for serum hepatitis. In only 1 horse (case 17) was the stem cell inoculum available for testing, and although this sample was EqPV-H positive, transmission via this method remains suppositional. If the stem cell inoculation was responsible for EqPV-H transmission, the contamination might have occurred from either EqPV-H infection of the stem cells themselves or carryover of donor serum used to culture the stem cells. Viral testing results of stem cell donor horses, albeit more than 14 weeks distant from the inoculation of cases 8 and 12, did not support transmission of EqPV-H by this route. Although the incubation time in these 2 cases was typical for serum hepatitis, EqPV-H infection might have occurred by another method.

The virologic testing in our study clearly links EqPV-H, but not the flaviviruses, with serum hepatitis. We found no evidence that infection or coinfection with the other known hepatotropic virus, NPHV, was associated with clinical disease. Although an original study by 2 of the current authors (T.J.D., B.C.T.) and others found an association between TDAV and plasma-associated hepatitis in a group of horses,¹³ the prospective study described here could not find TDAV in any of these field cases. Importantly, retrospective analysis of the commercial plasma botulinum antitoxin and experimental pony infection samples from the 2011 outbreak of Theiler's disease in which TDAV was discovered¹³ showed that EqPV-H was also present in the antitoxin, in diseased horses on the farm, and in the 4 experimental horses inoculated with the same plasma antitoxin lot.³⁰ Parvovirus was likely not detected in the original investigation because sequencing in that study focused on RNA viruses with proximity to hepatitis C virus, and so a DNAase treatment was performed on the RNA pellet before sequencing.¹³ Although TDAV and EPgV nucleic acids have been found in commercial plasma and serum products,^{13,29} pegiviruses are neither believed to be hepatotropic nor have they been documented to cause liver disease in any mammalian species.^{17,26,37} Our findings also suggests that they are rarely transmitted via TAT administration.

Epidemiologic data regarding Theiler's disease and virologic testing for EqPV-H are both consistent with the theory that subclinical or silent infection is likely common. This is supported by the low incidence of clinical disease after inoculation with the same biologic product.^{1,4,11,13} In addition, subclinical disease has been documented in multiple horses in 2 studies of biologic-associated serum hepatitis outbreaks,^{11,13} and in one of these outbreaks, disease was demonstrated to be associated with EqPV-H.^{13,30} Similarly, 2 horses experimentally inoculated with EqPV-H developed only mild clinical or subclinical disease.³⁰ Finally, a seroprevalence survey of 100 horses

found that 13% of horses without biochemical evidence of liver disease were EqPV-H positive,³⁰ suggesting that most horses that become infected with EqPV-H do not develop clinical disease. Taken together, these findings suggest that many horses infected with EqPV-H could have a short period of subclinical disease followed by complete recovery. Why some horses develop severe and often fatal disease after EqPV-H infection and others do not is unknown. Hepatic cell damage related to the high level of viremia and direct cytopathic effects is one possibility. Alternatively, injury might result as an indirect consequence of the immune response directed against the virus or injured hepatocytes, as occurs with hepatitis B virus in people.³⁸ The lymphocytic infiltration seen in many of these cases and in the experimentally EqPV-H-infected horses³⁰ could be consistent with an immune-mediated mechanism of liver damage.

Taken together, this prospective study lends additional support for a causative association between EqPV-H and equine serum hepatitis. This information should encourage blood product manufacturers to eliminate EqPV-H-infected horses from their facilities.

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CONFLICT OF INTEREST DECLARATION

Melissa Laverack, Randall Renshaw, and Edward Dubovi are employees of the New York Animal Health Diagnostic Center where equine hepatitis panel PCR testing is offered as fee-for-service. These authors were instrumental in viral testing development, validation, and performance but did not contribute to the specific analysis of results. Joy E. Tomlinson received speaker honoraria for presenting parts of this data at the 2018 ACVIM Forum, Seattle, Washington.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

All work was approved by the Cornell University IACUC #2012-0154.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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