

## **Specific Theiler's disease case and in-contact horse data by property**

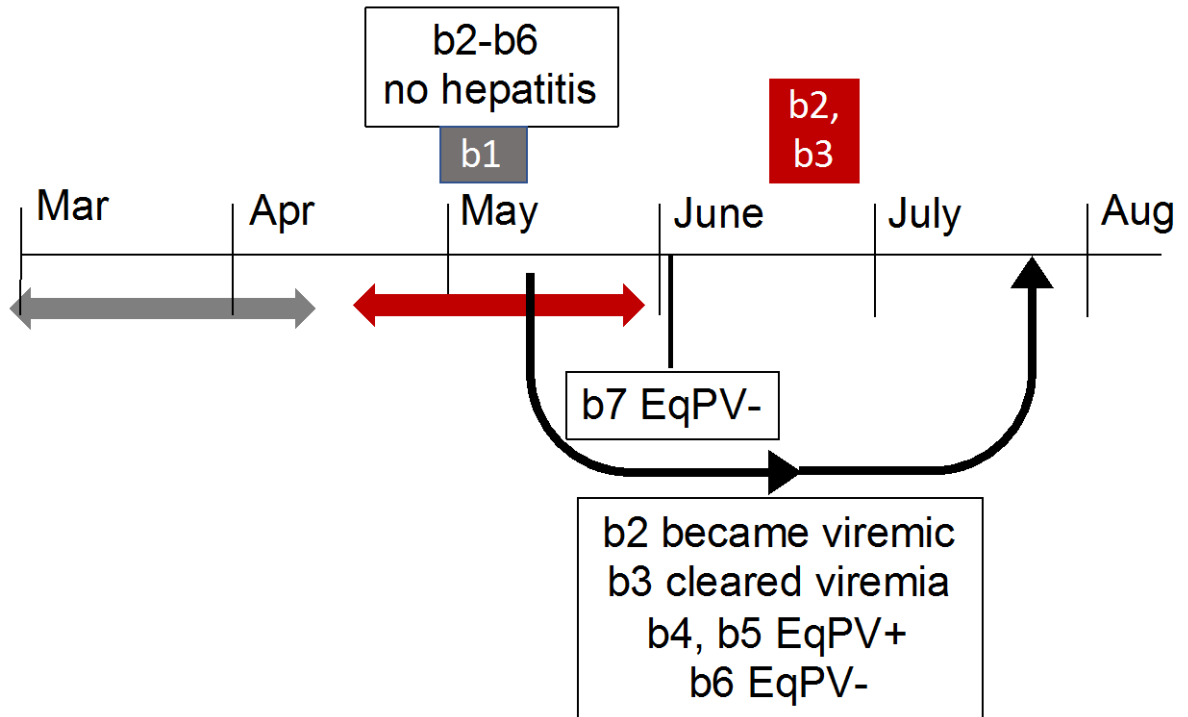
### *Property A*

A 12 y.o., 150 day-pregnant Arabian mare in Missouri (case a1) was diagnosed with Theiler's disease on September 21, 2015. The mare was euthanized the following day and both serum and liver samples were EqPV-H PCR+. No in-contact horses were tested.

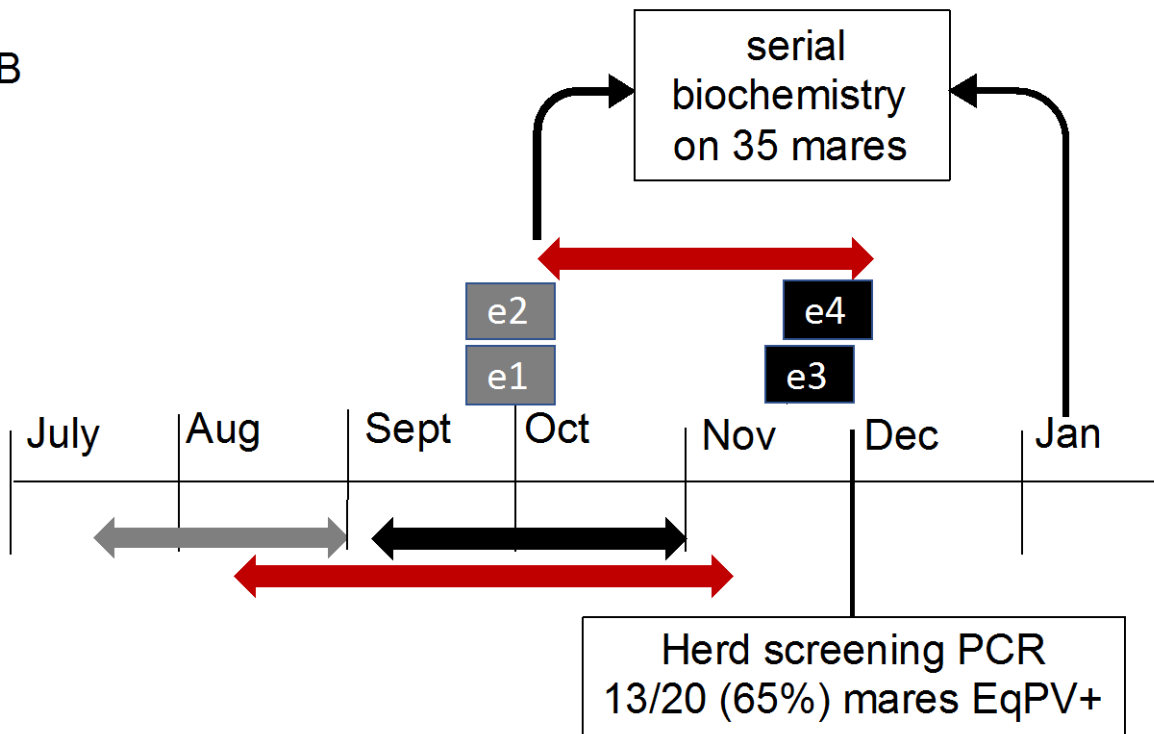
### *Property B*

A 12 y.o. Quarter Horse gelding in Kansas (case b1) was diagnosed with Theiler's disease on May 6, 2015 (Supplemental Figure 1). The gelding was euthanized on May 9<sup>th</sup> and a liver sample was EqPV-H PCR+. There were 6 in-contact horses (b2-7) that were screened by plasma PCR at varying time points (Supplemental Figure 1A, Supplemental Table 3). Horse b2 was EqPV-H PCR- and had normal serum chemistry at first test, developed moderate hepatitis 6 weeks later, and was EqPV-H PCR low+ 4 weeks after the episode of hepatitis. A predicted exposure period of 4-10 weeks before onset of hepatitis suggests this horse could have become infected after the first PCR. Horse b3 was EqPV-H PCR+ and had normal serum chemistry at first test, developed moderate hepatitis 6 weeks later, and was EqPV-H PCR negative 4 weeks after the episode of hepatitis. Horses b4 and b5 remained EqPV-H PCR+ during the 2 months of monitoring without any clinical signs of hepatitis. Horses b6 and b7, which were EqPV-H PCR-, showed no clinical signs during the observation period. Horses b4-b6 had normal serum chemistry at first PCR test.

A



B



Supplemental Figure 1: Timeline of herd testing after cases of Theiler's disease on Property B in Kansas (A) and Property E in New Jersey (B). Cases of Theiler's disease are indicated in gray or black boxes above the timeline. The time of diagnosis of additional cases of clinical or subclinical hepatitis is indicated in red above the timeline. Predicted exposure periods for each respective set of cases are shown below the timeline. Serum PCR data for in-contact horses is displayed below the timeline. (A) After the index case on Property B, b1, was euthanized, 5 in-contact horses were screened for hepatitis (serum chemistry) and EqPV-H (serum PCR) in May. No horses had hepatitis at that time, although 3 (b3, b4 and b5) were PCR+. Three in-contact horses (b2, b3 and b4) developed non-specific signs of illness in late June and two (b2 and b3) were confirmed to have biochemical evidence of hepatitis at that time. The 5 horses were re-tested for EqPV-H in July. A sixth in-contact horse was tested by PCR in June but did not have serum chemistry performed. (B) After 2 index cases of Theiler's disease (e1 and e2) were euthanized at the end of September, the farm instituted serial serum biochemistry screening of 35 mares. Cases of mild clinical or subclinical hepatitis were diagnosed between the beginning of October and early December (red arrows above the timeline), although monitoring continued into January. Two additional cases of Theiler's disease (e3 and e4) developed among these 35 monitored mares. EqPV-H PCR was performed on 20 mares at a single timepoint in early December.

Supplemental Table 3: Clinical data for the horses from Property B. QH, Quarter Horse; G, gelding; F, female; ND, not detected; Ct, cycle threshold (i.e. number of cycles required for the fluorescent signal to exceed background level). On May 12, horses b2-b6 all had normal serum chemistry.

Case	b1	b2		b3		b4		b5		b6		b7
<b>Age (years)</b>	12	12		7		6		17		15		15
<b>Breed</b>	QH	QH		QH		QH		QH		QH		QH
<b>Sex</b>	G	F		G		G		G		G		F
<b>PCR</b>												
<b>Sample</b>	liver	plasma		plasma		plasma		plasma		plasma		plasma
<b>Date</b>	May 9	May 12	July 20	May 12	July 20	May 12	July 20	May 12	July 20	May 12	July 20	June 12
<b>EqPV-H (Ct)</b>	19.79	ND	36.2	25.54	ND	30.13	32.2	35.99	36.1	ND	ND	ND
<b>NPHV (Ct)</b>	ND	ND	ND	34.42	ND	ND	ND	ND	ND	ND	ND	ND
<b>TDAV (Ct)</b>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>EPgV (Ct)</b>	32.09	ND	ND	ND	ND	ND	ND	25.92	27.84	ND	ND	ND
<b>Chemistry date</b>	May 6	June 25		June 25		June 25		n/a		n/a		n/a
<b>GGT</b> 5-30 U/L	<b>108</b>	<b>182</b>		<b>50</b>		16						
<b>ALP</b> 20-275 U/L	255	<b>829</b>		<b>293</b>		189						
<b>LDH</b> 150-450 U/L	<b>1615</b>	<b>805</b>		<b>654</b>		<b>470</b>						
<b>tBili</b> 0.1-2.5 mg/dL	<b>21</b>	<b>9.2</b>		1.3		0.9						

### *Property C*

A 7 y.o. Quarter Horse gelding in Missouri (case c1) was diagnosed with Theiler's disease on July 30, 2017. The gelding died August 1<sup>st</sup> and a liver sample was EqPV-H PCR+.

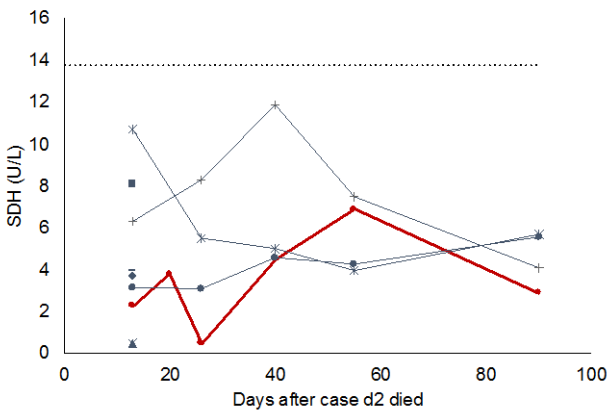
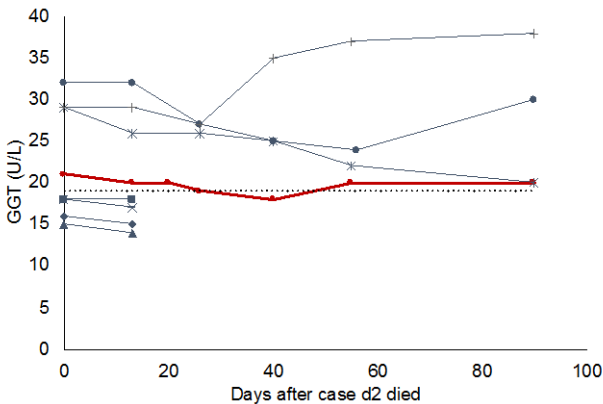
Case c1 had been preceded by a case of mild clinical hepatitis in another horse on the property (case c2) on July 21<sup>st</sup> that survived with no treatment beyond administration of ceftiofur. In addition to c2, 4 more in-contact horses developed non-specific clinical signs of illness and 2/3 had evidence of hepatitis on serum chemistry, 1 had normal liver enzymes, and the last did not have serum chemistry performed (Supplemental Table 4). All 6 horses tested from this property were EqPV-H PCR+ (Supplemental Table 4).

Supplemental Table 4: Clinical data for the horses from Property C. QH, Quarter Horse; G, gelding; F, female; ND, not detected; Ct, cycle threshold (i.e. number of cycles required for the fluorescent signal to exceed background level).

<b>Case</b>	<b>c1</b>	<b>c2</b>	<b>c3</b>	<b>c4</b>	<b>c5</b>	<b>c6</b>	
<b>Age</b>	7	4	12	6	6	3	
<b>Breed</b>	QH	QH	QH	QH	QH	QH	
<b>Sex</b>	G	F	G	G	G	F	
<b>PCR</b>							
<b>Sample</b>	liver	serum	serum	serum	serum	serum	
<b>Sample date</b>	1-Aug	4-Sep	4-Sep	4-Sep	4-Sep	21-Sep	
<b>EQPV-H (Ct)</b>	22	37.3 (suspect)	28.6	31.9	36.5	29	
<b>Chemistry</b>							
<b>Sample date</b>	30-Jul	21-Jul	23-Aug	23-Aug	23-Aug	4-Aug	not done
<b>GGT</b> 2-36 U/L	<b>137</b>	<b>146</b>	<b>70</b>	14	<b>82</b>	<b>126</b>	
<b>AST</b> 148-420 U/L	<b>3953</b>	<b>&gt;1000</b>	339	175	304	318	
<b>tBili</b> 0.4-2.8 mg/dL	<b>20.1</b>	<b>18.8</b>	1	1.1	1.2	0.9	
<b>dBili</b> 0-0.5 mg/dL	<b>2.3</b>	n/a	0.3	0.4	0.4	0.3	
<b>CK</b> 45-360 U/L	<b>1389</b>	<b>724</b>	189	111	143	249	
<b>Glucose</b> 68-120 mg/dL	<b>10</b>		87	88	<b>64</b>	100	

*Property D*

A 20-year-old Welsh Pony gelding in North Carolina (case d1) developed Theiler's disease May 14, 2017. The gelding was euthanized the following day and serum and liver samples were EqPV-H PCR+. Two weeks later, a second pony on the same property, case d2, also developed fatal Theiler's disease and was euthanized. Case d2 was EqPV-H+ on serum, but no liver sample was available. Subsequently, the owner of the 2 ponies elected to have the remaining 9 horses on the property screened by serum chemistry and EqPV-H PCR (Table 2, Supplemental Figure 2). Only 1 pony was EqPV-H+. Four ponies had serum GGT above reference interval (4-19 U/L) although all were < 35 U/L at the time of PCR and all horses remained clinically healthy.



Supplemental Figure 2: Nine horses in contact with cases d1 and d2 were monitored by serial serum GGT, SDH, total bilirubin, and bile acids for 3 months following the death of case d2. GGT and SDH are shown. Total bilirubin and bile acids remained within reference interval in all horses at all timepoints. At day zero, all 9 horses were screened for EqPV-H by serum PCR and only one horse, denoted in red, was PCR positive. The upper limit of the reference interval is denoted by the horizontal dotted line.

### *Property E*

A 5-year-old pregnant Standardbred mare in New Jersey (case e1) developed Theiler's disease September 29, 2015 and was euthanized within 24 hours. The following day, September 30, a 16-year-old pregnant Standardbred mare (case e2) also developed Theiler's disease and was euthanized two days later. Liver samples from both these animals were EqPV-H PCR+. The owner of this farm monitored a large number of in-contact horses from October 2015 to January 2016. These results are described in the next paragraph and presented in Supplemental Figure 1B and Supplemental Tables 4 and 5. During this monitoring period, two monitored horses developed Theiler's disease: case e3 on November 19, 2015 and case e4 on November 28, 2015. Both mares died or were euthanized within hours of detecting clinical signs and liver samples from both were also EqPV-H PCR+.

Including cases e3 and e4, 35 broodmares were monitored for hepatitis by serum chemistry between October 6<sup>th</sup> 2015 and January 16<sup>th</sup> 2016 (Supplemental Figure 1B). Of those mares, 18 (51%) developed hepatitis defined as GGT > 35 U/L (the upper limit of the laboratory reference interval) during this time, including 7 (20%) with acute hepatitis as evidenced by concurrent increase in both AST and GGT above the upper limit of reference intervals. Excluding cases e3 and e4, the 16 mares with hepatitis had a median maximal GGT of 46 U/L (range 37 – 116 U/L) with maximal AST of 351 U/L (249 – 2489 U/L). Only cases e3 and e4 showed clear clinical signs. Five mares with subclinical disease were treated with supportive care and all survived. The remaining 11 mares with hepatitis also survived.

Twenty of the monitored mares (not including cases e1, 2, 3 and 4) were tested for EqPV-H by serum PCR in December and 13 (65%) were positive. The hepatitis status of the 20 mares at the time of PCR is summarized in Supplemental Table 5. The subset of 20 horses that were

screened by PCR did not include all mares that had hepatitis at some point during the monitoring period. The EqPV-H status of all 20 mares with hepatitis on the property (including cases e1-4) is summarized in Supplemental Table 5.

Supplemental Table 5: Hepatitis status of 20 horses screened for EqPV-H by serum PCR after 4 herd-mates died of Theiler’s disease. Hepatitis was classified as “Active” if GGT was > 35 U/L within 2 weeks of the PCR; “Resolved” if GGT had been > 35 U/L within the prior 2 months but was normal at the time of PCR; and “Not detected” if GGT was < 35 U/L at all times tested within the previous 2 months.

No. cases	EqPV-H PCR	Hepatitis		
		Active	Resolved	Not detected
13	positive	4 (31%)	4 (31%)	5 (38%)
7	negative	0	4 (57%)	3 (43%)

Supplemental Table 5: EqPV-H status of 20 horses that developed biochemical evidence of liver disease between September and December of 2015, including 4 cases that died of Theiler’s disease. Hepatitis was classified as “Active” if GGT was > 35 U/L within 2 weeks of the PCR or “Resolved” if GGT had been > 35 U/L within the preceding 2 months but was normal at the time of PCR.

No. cases	Hepatitis status at time of PCR	EqPV-H PCR+
8	active	8 (100%)
8	resolved	4 (50%)
4	n/a	not done



### *Property F*

A 7 y.o., Arabian gelding in Washington (case f1) was diagnosed with Theiler's disease on October 22, 2017. The gelding was euthanized the same day. A frozen liver sample was EqPV-H PCR negative.

### Discussion of supplemental material

While the timing of serum chemistry and PCR for the in-contact horses at Properties B and E was not ideal, the findings remain compatible with the theory that EqPV-H could be causative of the hepatitis on these properties. On these properties, there were two situations where in-contact horses had hepatitis but were EqPV-H negative. First, case b2 tested EqPV-H negative and subsequently developed hepatitis. Assuming a similar 4-10 week incubation period as seen with biologic product associated cases of Theiler's disease<sup>1,4,8</sup>, a predicted exposure period for case b2 would be approximately mid-April to the end of May (Supplemental Figure 1A). This timing could be consistent with horse b2 becoming infected shortly after the first PCR screen. Second, multiple horses on Property E had hepatitis that had resolved before the PCR was performed. The experimental infections with EqPV-H performed to date demonstrate that EqPV-H viremia drops after hepatitis and could be cleared<sup>18</sup>.

Some horses on Property D had hepatitis, as indicated by serum GGT activities above the upper limit of the specific laboratory's reference interval, and were EqPV-H negative. This laboratory utilized a lower maximum reference value than the other laboratories in this study, and, at the time of PCR, all horses had GGT < 35 U/L which is a commonly utilized maximum value for other laboratories.. Therefore, the significance of GGT values in these horses is unclear. However, it is possible that another factor, such as a feed contaminant, contributed to

liver disease on this property, or that the timing of the PCR test was inappropriate to identify horses during a viremic phase.