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Environmental persistence of equid herpesvirus type-1

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Abstract

Background: Equid herpesvirus type 1 (EHV-1) is ubiquitous in equine populations causing respiratory disease, and complications including late-term abortion and neurological disease. Eradication of EHV-1 from housing environments that typically contain unsealed wood and porous bedding materials can be challenging. However, consideration should be given to take advantage of the viral envelope's susceptibility to environmental conditions.

Objective: To determine environmental persistence of EHV-1 on materials and in environmental conditions commonly found in equine facilities. We hypothesised that environmental conditions and materials would limit environmental persistence of EHV-1 in horse housing environments.

Study design: Experimental study.

Methods: Standard inoculum of EHV-1 strain OH03 was applied to leather, polyester-cotton fabric, two bedding materials (pinewood shavings and wheat straw) and polystyrene (plastic), and placed under three different environmental conditions (4°C, indoors and outdoors). Virus titration and quantitative PCR (qPCR) were performed at six time points between 0 and 48 hours and the number of plaque-forming units (PFUs) was determined.

Results: Viable EHV-1 was recovered up to 48 hours from all material-environmental condition combinations, with persistence decreasing over time. In general, outdoor environment had the greatest impact, irrespective of material tested, followed by indoor environment and 4°C. On average, wood shavings had the greatest impact on persistence, followed by leather, straw, fabric and polystyrene.

Main limitations: The inoculum used in this study was not in a milieu consistent with nasal secretions. As such, virus particles may have been more sensitive to the materials and/or environmental conditions evaluated.

Conclusions: Environmental factors had variable effects on environmental persistence. Although there were significant reductions in PFUs within the first 3 hours, irrespective of environment-material evaluated, viable virus was still recovered at 48 hours likely representing a transmission risk. Barrier precautions should be used to prevent spread of EHV-1 from unrecognised environmental reservoirs.

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KEYWORDS
horses, infection control, biosecurity

1 | INTRODUCTION

Equid herpesvirus type 1 (EHV-1) is ubiquitous in the equine population—affecting horses of every breed and discipline. While the majority of horses have been exposed by 1 year of age, and typically experience mild respiratory disease, cell-associated viraemia may lead to more serious complications including late-term abortion, neonatal foal death and neurological disease (equine herpes myeloencephalopathy or EHM).[1] In addition, infected horses develop a latent infection which has the potential to recrudesce in periods of stress. Many outbreaks are reported yearly in multiple states in the United States, as is evidenced by the US Equestrian Federation Equine Disease Communication Center Outbreak Alerts.[2]

As EHV-1 is ubiquitous in the equine population, it is unrealistic to consider eradication as a means of control. Rather, we need to effectively manage this agent in populations based on epidemiological factors that likely contribute to the development of disease. In naturally occurring EHM, EHV-1 can be shed intermittently and the amount of DNA detected in nasal swabs can vary markedly over time, irrespective of the severity of disease.[3] This equates to the potential for significant nasal shedding of this virus and widespread environmental contamination to occur before infections are recognised.

As a member of the alphaherpesvirinae, EHV-1 has a viral envelope, containing specific glycoproteins necessary for host cell penetration, which must remain intact for its environmental survival, transmission and infection. As an enveloped virus, it is susceptible to many commonly used disinfectants as well as environmental conditions such as air temperature, desiccation and ultraviolet (UV) light. Despite this, it can still be challenging to eradicate infectious agents, such as EHV-1, from typical horse housing environments that generally contain unsealed wood and porous bedding materials (eg wood shavings, straw) in areas that are often protected from UV light exposure. Thus, we should not rely solely on cleaning and disinfection as the only means of controlling EHV-1 in equine populations and facilities. Consideration should be given to delaying repopulation of facilities when there is widespread contamination to take advantage of this virus' natural weaknesses, namely the susceptibility of its envelope to environmental conditions.

To date, there is very limited research to indicate the duration of environmental persistence of EHV-1 in the barn environment. There is a single experimental study from 1959 evaluating the persistence of a single strain (Ky-D) on relevant materials including straw, wood and rope; reporting persistence of 7-42 days, depending on the material tested.[4] It is important to note, that while this study does provide some evidence regarding environmental persistence, it does so by evaluating a single strain, in an unreported number of samples, in a laboratory setting. Given these limitations, it is difficult to draw conclusions on which to base decisions in the field. Developing a strong foundation to make clinical decisions relies upon the ability to not just characterise or demonstrate an association in a single instance, but also necessarily needs to be consistently repeatable; and, when considering the management of diseases in populations, ideally demonstrated in the environment in which it will be applied.

With this in mind, the objective of this study was to determine the environmental persistence of EHV-1 on materials and in environmental conditions commonly found in equine facilities. We hypothesised that different combinations of environmental conditions and materials commonly used in horse housing environments would limit environmental persistence of EHV-1.

2 | MATERIALS AND METHODS

2.1 | Study overview

An experimental in vitro study was conducted to evaluate the environmental persistence of a wild-type EHV-1 strain on materials commonly found in the equine housing environment. A standard inoculum of EHV-1 strain OH03 was applied to leather, polyester-cotton fabric, two bedding materials (pinewood shavings and wheat straw) and polystyrene (plastic), and placed under three different environmental conditions (4°C, indoors and outdoors). A virus titration and quantitative PCR (qPCR) were performed at six time points between 0 and 48 hours (t = 0, 3, 12, 24, 36 and 48 hours). The number of plaque-forming units (PFUs) as well as EHV-1 genome copy numbers were determined.

2.2 | EHV-1 inoculum preparation

This experiment used EHV-1, strain Findlay OH03, donated by Dr Klaus Osterrieder (Cornell University), propagated in Kentucky equine dermal cells (KyED; ATCC CCL-57). Virus-laden supernatant was generated by growing KyED to 80%-90% confluence in minimal essential media (MEM, GIBCO), washed with phosphate-buffered saline (PBS, GIBCO) and then infected with 100 μL of approximately 7.33×10^7 PFU/mL. Flasks were incubated for 24-48 hours at 37°C until 90% cytopathic effect (CPE) was observed at which point supernatants were collected, cellular debris was removed by centrifugation and viral titres were determined. Inoculum used in this experiment was made by creating a 1×10^6 viral suspension by adding 200 μL of stock solution (1×10^7 PFU/mL) into 20 mL of bronchoalveolar lavage (BAL) supernatant fluid derived as left-over from a diagnostic procedure. Fluid was tested for EHV-1 and EHV-4 via qPCR.

TABLE 1 Least squares mean $\log_{10}$ titre (PFU/mL) of EHV-1 recovered from various material and environmental condition combinations

Surface	Storage	Time	Estimate	95% LCL	95% UCL	% Reduction
Polystyrene	Baseline	0	4.94	4.77	5.12	
	4°C	3	4.86	4.29	5.44	1.5%
		12	4.90	4.33	5.48	0.7%
		24	4.77	4.20	5.34	3.5%
		36	4.58	4.00	5.15	7.4%
		48	3.72	3.15	4.29	24.7%
	Indoor	3	5.02	4.53	5.51	−1.6%
		12	4.94	4.45	5.44	−0.1%
		24	4.79	4.30	5.29	3.0%
		36	4.59	4.10	5.09	7.0%
		48	4.41	3.92	4.90	10.7%
	Outdoor	3	5.23	4.73	5.72	−5.8%
		12	4.64	4.14	5.13	6.2%
		24	3.68	3.19	4.17	25.5%
		36	3.10	2.61	3.59	37.3%
		48	2.83	2.33	3.32	42.8%
Polyester cotton fabric	Baseline	0	5.30	5.14	5.46	
	4°C	3	5.02	4.44	5.59	5.3%
		12	4.69	4.11	5.26	11.6%
		24	4.52	3.95	5.10	14.6%
		36	4.12	3.55	4.69	22.3%
		48	3.75	3.18	4.33	29.2%
	Indoor	3	4.90	4.40	5.39	7.6%
		12	4.66	4.17	5.16	12.0%
		24	4.20	3.71	4.70	20.7%
		36	3.92	3.43	4.41	26.1%
		48	3.59	3.09	4.08	32.4%
	Outdoor	3	4.75	4.26	5.25	10.3%
		12	3.45	2.95	3.94	35.0%
		24	1.73	1.23	2.22	67.4%
		36	0.82	0.33	1.32	84.4%
		48	0.16	−0.33	0.65	97.0%
Leather	Baseline	0	3.71	3.56	3.86	
	4°C	3	3.00	2.43	3.57	19.1%
		12	2.83	2.26	3.41	23.6%
		24	2.94	2.37	3.51	20.7%
		36	2.72	2.15	3.30	26.6%
		48	2.75	2.17	3.32	26.0%
	Indoor	3	2.79	2.30	3.28	24.9%
		12	2.01	1.52	2.50	45.8%
		24	1.47	0.98	1.96	60.4%
		36	1.86	1.37	2.36	49.8%
		48	1.58	1.08	2.07	57.5%
	Outdoor	3	2.69	2.19	3.18	27.6%
		12	2.28	1.78	2.77	38.6%
		24	0.94	0.45	1.44	74.6%
		36	0.68	0.19	1.17	81.6%
		48	1.50	1.01	1.99	59.6%
Wheat straw	Baseline	0	5.08	4.79	5.37	
	4°C	3	4.46	3.89	5.03	12.2%
		12	4.34	3.76	4.91	14.7%
		24	3.74	3.16	4.31	26.5%
		36	4.21	3.63	4.78	17.2%
		48	3.35	2.78	3.92	34.0%
	Indoor	3	4.49	4.00	4.98	11.7%
		12	4.02	3.52	4.51	20.9%
		24	3.50	3.01	3.99	31.1%
		36	2.78	2.29	3.27	45.3%
		48	2.12	1.63	2.61	58.2%
	Outdoor	3	3.82	3.33	4.31	24.8%
		12	2.54	2.05	3.03	50.0%
		24	2.41	1.91	2.90	52.7%
		36	1.43	0.94	1.92	71.9%
		48	1.16	0.66	1.65	77.2%
Wood shavings	Baseline	0	2.84	2.39	3.30	
	4°C	3	0.92	0.35	1.50	67.5%
		12	0.73	0.16	1.30	74.3%
		24	0.84	0.26	1.41	70.5%
		36	0.54	−0.03	1.11	81.0%
		48	0.61	0.04	1.18	78.5%
	Indoor	3	1.03	0.53	1.52	63.8%
		12	0.99	0.50	1.49	65.0%
		24	1.05	0.56	1.54	63.0%
		36	0.58	0.09	1.07	79.6%
		48	0.66	0.16	1.15	76.9%
	Outdoor	3	0.82	0.33	1.32	71.0%
		12	0.58	0.09	1.08	79.5%
		24	0.44	−0.05	0.94	84.4%
		36	0.37	−0.12	0.86	86.9%
		48	0.37	−0.12	0.86	86.9%
Inoculum	n/a	n/a	6.03	5.68	6.37	n/a

2.3 | Materials preparation and inoculation

Five materials commonly found in equine housing environments were selected for evaluation in this study including leather (1 × 1 cm), polyester-cotton fabric (1 × 1 cm), pine wood shavings bedding (1 g), wheat straw bedding (1 g) and polystyrene/plastic (flat bottom well tissue culture plate). Materials were cut to size

or weighed, irradiated via microwave treatment (10 minutes) and placed in 24-well polystyrene tissue culture plates in duplicate, leaving two empty wells on each polystyrene plate. Materials were inoculated with 200 μL of virus inoculum (described above) with the plate for t = 0 prepared after all other plates were distributed to their respective locations (described below). A plate with only PBS served as a control.

All materials were evaluated under three different environmental conditions, including: (a) 4°C with no light exposure (placed in a laboratory refrigerator); (b) outdoor environment (rooftop; 7 AM or 7 PM start time); and, (c) indoor environment (barn; 7 AM or 7 PM start time). All experiments were repeated back-to-back and conducted in May 2012 at the James L Voss Veterinary Teaching Hospital (JLV-VTH) at Colorado State University. Rooftop placement of outdoor samples was required to meet Institutional Biosafety Committee requirements.

2.4 | Sample collection and processing

For each experiment (7 AM or 7 PM start time), and for each environmental condition (4°C, outdoor and indoor), a total of six plates were prepared. This allowed sampling at six time points: t = 0, 3, 12, 24, 36 and 48 hours. The culture plates for each experiment were secured in transparent, plastic containers with lids. The container sidewalls had several 2 cm diameter openings for ventilation, and clasps on the side for a tight lid closure. Plates in the outdoor environment were put in a cage and placed in a shaded area of the JLV-VTH rooftop. Plates in the indoor environment were put in a cage in an unheated but populated (sheep) barn on the JLV-VTH grounds. All containers were fitted with calibrated thermometers for ambient temperature data collection.

At each time point (t = 0, 3, 12, 24, 36 or 48 hours), a plate was collected, the ambient temperature was recorded, and brought back to

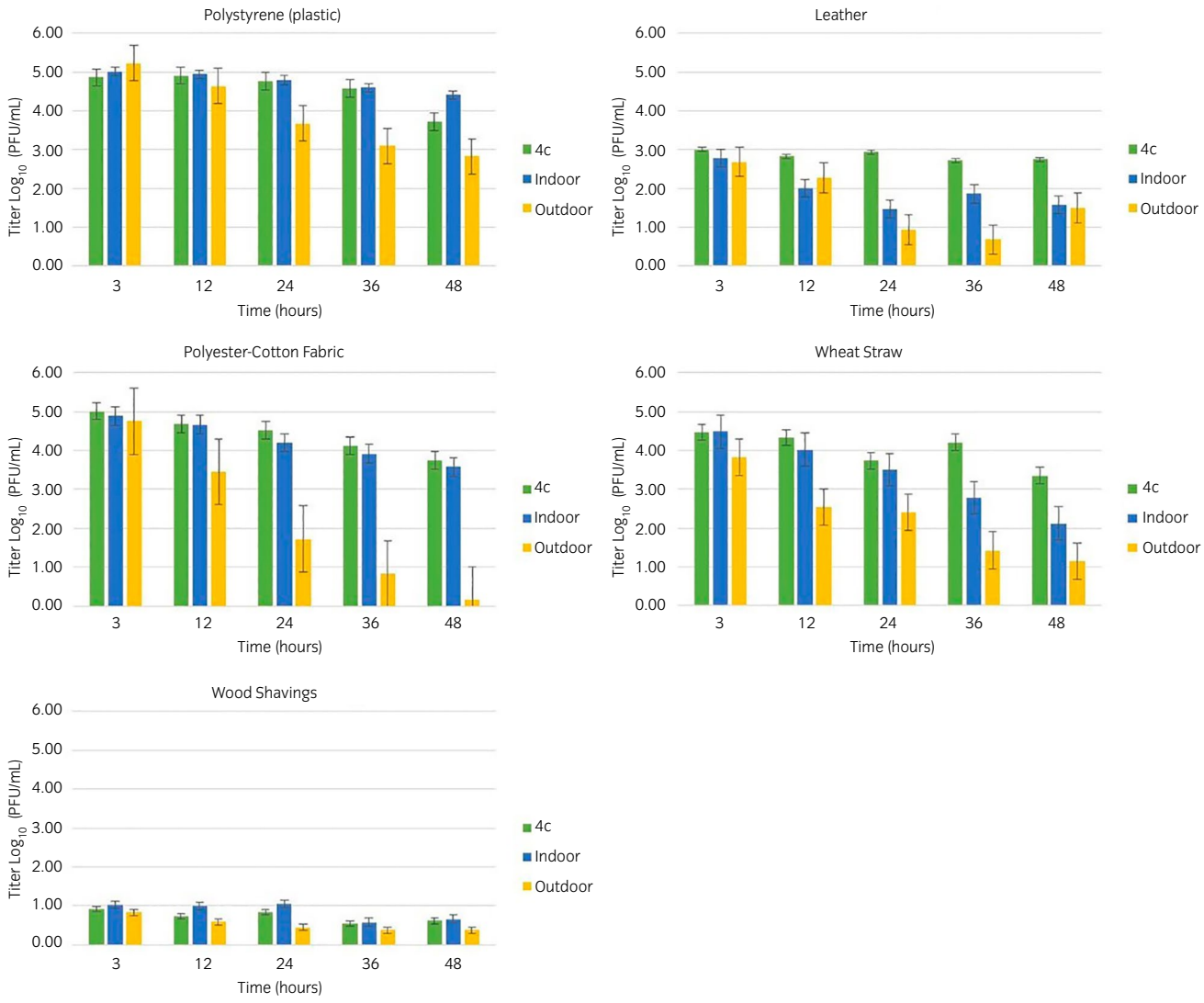

FIGURE 1 Least squares means $\log_{10}$ titre of EHV-1 recovered from different environment-material combinations over time

TABLE 2 Comparison of least squares mean (log_{10}) of viable (PFU/mL) and total EHV-1 DNA (gB copies/mL) from various material and environmental condition combinations

Condition			Mean PFU/mL (log_{10})			Mean gB Copies (log_{10})		
Surface	Storage	Time	Estimate	95% LCL	95% UCL	Estimate	95% LCL	95% UCL
Polystyrene	Baseline	0	4.94	4.77	5.12	4.32	3.83	4.80
	4C	12	4.90	4.33	5.48	3.66	3.09	4.24
		24	4.77	4.20	5.34	3.22	2.64	3.79
		48	3.72	3.15	4.29	3.85	3.28	4.43
	Indoor	12	4.94	4.45	5.44	3.88	3.39	4.36
		24	4.79	4.30	5.29	3.63	3.14	4.11
		48	4.41	3.92	4.90	3.74	3.26	4.23
	Outdoor	12	4.64	4.14	5.13	3.95	3.47	4.43
		24	3.68	3.19	4.17	3.75	3.27	4.24
		48	2.83	2.33	3.32	3.01	2.53	3.49
Polyester cloth fabric	Baseline	0	5.30	5.14	5.46	3.74	3.26	4.22
	4°C	12	4.69	4.11	5.26	3.68	3.11	4.25
		24	4.52	3.95	5.10	3.82	3.25	4.40
		48	3.75	3.18	4.33	3.83	3.26	4.40
	Indoor	12	4.66	4.17	5.16	3.94	3.46	4.42
		24	4.20	3.71	4.70	3.90	3.41	4.38
		48	3.59	3.09	4.08	3.96	3.48	4.44
	Outdoor	12	3.45	2.95	3.94	3.67	3.19	4.15
		24	1.73	1.23	2.22	3.80	3.32	4.28
		48	0.16	−0.33	0.65	3.09	2.61	3.57
Leather	Baseline	0	3.71	3.56	3.86	1.43	1.00	1.86
	4°C	12	2.83	2.26	3.41	1.00	0.33	1.67
		24	2.94	2.37	3.51	0.87	0.39	1.35
		48	2.75	2.17	3.32	0.72	0.04	1.39
	Indoor	12	2.01	1.52	2.50	1.89	1.41	2.37
		24	1.47	0.98	1.96	1.07	0.56	1.58
		48	1.58	1.08	2.07	1.98	1.53	2.44
	Outdoor	12	2.28	1.78	2.77	1.66	0.99	2.32
		24	0.94	0.45	1.44	1.34	0.89	1.80
		48	1.50	1.01	1.99	0.83	0.35	1.32
Wheat straw	Baseline	0	5.08	4.79	5.37	3.52	3.04	4.00
	4°C	12	4.34	3.76	4.91	3.20	2.63	3.78
		24	3.74	3.16	4.31	3.04	2.47	3.61
		48	3.35	2.78	3.92	2.61	2.04	3.18
	Indoor	12	4.02	3.52	4.51	2.77	2.29	3.25
		24	3.50	3.01	3.99	2.75	2.27	3.23
		48	2.12	1.63	2.61	2.29	1.81	2.77
	Outdoor	12	2.54	2.05	3.03	2.62	2.13	3.10
		24	2.41	1.91	2.90	2.56	2.10	3.02
		48	1.16	0.66	1.65	2.46	1.95	2.98

(Continues)

TABLE 2 (Continued)

Condition			Mean PFU/mL (log_{10})			Mean gB Copies (log_{10})		
Surface	**Storage**	**Time**	**Estimate**	**95% LCL**	**95% UCL**	**Estimate**	**95% LCL**	**95% UCL**
Wood shavings	Baseline	0	2.84	2.39	3.30	3.37	2.89	3.85
	4°C	12	0.73	0.16	1.30	3.68	3.11	4.25
		24	0.84	0.26	1.41	3.30	2.73	3.88
		48	0.61	0.04	1.18	3.39	2.81	3.96
	Indoor	12	0.99	0.50	1.49	3.37	2.88	3.85
		24	1.05	0.56	1.54	3.40	2.92	3.89
		48	0.66	0.16	1.15	3.19	2.70	3.67
	Outdoor	12	0.58	0.09	1.08	3.06	2.58	3.54
		24	0.44	−0.05	0.94	3.08	2.59	3.56
		48	0.37	−0.12	0.86	2.54	2.06	3.02
Inoculum	n/a	n/a	6.03	5.68	6.37	5.65	5.17	6.13

the laboratory for processing. For each sample well, fluid was collected (the sample), the well was rinsed with 1.8 mL MEM-10 and added to the corresponding sample. Tubes were vortexed for 60 seconds, centrifuged (500 *g*/5 min) and the supernatant was stored at −80°C until further analysis.

2.5 | Virus titration and virus genomic DNA quantification

Samples underwent virus titration via plaque counts. Rabbit kidney cells (RK13) were grown to confluence in MEM-10 and seeded into wells of 12-well plates. Samples were serially diluted, and 100 μL were transferred onto RK13 cell layer and incubated at 37°C, CO_2 5% for 72 hours. Wells were stained with crystal violet (Fisher Scientific) and plaques were counted. Additionally, DNA was extracted from 1-mL aliquots of stored samples using a QIAGEN Blood Mini Kit (Qiagen) per manufacturer's directions. Quantitative real-time PCR analysis was performed to quantify the number of EHV-1 specific glycoprotein B (gB) genome copies per mL of the sample as previously described.[5]

2.6 | Environmental temperature and relative humidity

Experiments were conducted in April and May 2012. All sample containers were fitted with calibrated thermometers for ambient temperature data collection and data regarding average environmental temperature and precipitation were obtained from the National Oceanic and Atmospheric Administration (NOAA).

2.7 | Data analysis

All data were evaluated using descriptive statistics. Least squares mean of gB copies and of plaque count, and 95% confidence intervals were derived from multivariable mixed models of log transformed titres for each combination of material-environment-time (ie a three-way interaction), accounting for repeated measures and the hierarchical nature of the data. All analyses were performed using commercially available software (SAS v9.4, SAS Inc.) with a *P*-value for significance set at ≤.05.

3 | RESULTS

In this study, viable EHV-1 was recovered up to 48 hours from all material-environmental condition combinations, with viral environmental persistence decreasing over time ($P < .001$) regardless of material ($P < .001$) or environment ($P < .001$) tested. In general, the outdoor environment had the greatest impact on persistence over time, irrespective of material tested, followed by indoor environment and 4°C (Table 1; Figure 1). On average, regardless of environment, wood shavings had the greatest impact on persistence, followed by leather, wheat straw, fabric and polystyrene.

During May 2012, the daily temperature ranged from 6.8°C (44.2°F) to 24.1°C (75.3°F) with an average daily temperature of 15.4°C (59.7°F) and an average of 1.61 inches of precipitation. There was no significant difference in environmental persistence when the experiment started in the evening (PM) or in the morning (AM); and detection of gB copies was stable throughout the observational time regardless of the material or the environment under investigation (Table 2). Interestingly, detection of gB copies was considerably lower for leather as compared to PFUs suggesting that leather may have either destroyed some of the DNA or may contain PCR inhibitors. Detection of gB copies was higher for wood shavings as compared to PFUs suggesting that it may be more difficult to recover EHV-1 from wood shavings or that the viral envelope was compromised resulting in an inability to replicate.

4 | DISCUSSION

This study found that while significant reductions in viable virus occurred on a variety of material-environmental condition combinations—EHV-1

persisted for up to 48 hours on all materials and environments tested. Although some of these PFU counts were very low for specific materials such as wood shavings, it is unknown if this level of contamination would result in infectious disease transmission. Experimentally, EHV-1–associated neurological disease is difficult to consistently and reliably induce. For example, previous experimental inoculations using EHV-1 field strain Findlay OH03 (the same strain used in this study) have resulted in minimal neurological signs when inoculating with 5×10^6 PFU in 5 mL via nebulisation[6] and no clinical signs when inoculating with 5×10^7 PFU in 4 mL via intranasal instillation.[7]

The immediate reduction of PFU on wood shavings was unexpected, but may be due in part from immediate viral envelope destruction and/or the result of viral particles being retained in the porous material during sample collection. Consistent detection of gB copies for all condition-time combinations evaluated suggests that this is a viability issue rather than a recovery issue with respect to wood shavings. While PFUs on leather consistently dropped over time as expected, there was a lower than expected detection of gB copies for all condition-time combinations evaluated, suggesting that there may have been some recovery issues and/or inhibition of the PCR relative to samples derived from this material.

This study had a few notable limitations. As all experiments were performed in the same 2-day period, we were unable to evaluate the impact of ambient conditions. Additionally, the inoculum was not in a milieu consistent with equine nasal secretions, as such, virus particles may have been more sensitive to the materials and environmental conditions evaluated than if they had been afforded 'protection' via nasal mucus. While this study suggests that EHV-1 virus particles may survive for at least 48 hours in the conditions tested, this may in fact be an underestimate. Finally, we were unable to follow this experiment to its natural conclusion of no viral recovery. As such, we are unable to provide recommendations for delayed repopulation of facilities where there has been significant widespread contamination.

This study confirms the findings of the 1959 report,[4] that different materials and environmental conditions can impact the environmental persistence of EHV-1, and demonstrates the importance for considering infection control in managing horse populations. Specifically, it is recommended to employ routine measures such as barrier nursing and rigorous cleaning and disinfection, to decrease the potential for widespread contamination and subsequent reservoir development.

CONFLICT OF INTERESTS

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

Study conceptualisation and overall supervision were provided by L.S. Goehring. Study execution and data collection were conducted by L.V. Ashton and N.T. Saklou with primary oversight by L.S. Goehring. B.A. Burgess and P.S. Morley conducted data analyses and interpretations, assisted by L.S. Goehring; with all authors having full access to study data. N.T. Saklou and B.A. Burgess are the primary authors of the manuscript with oversight by L.S. Goehring; all other authors had an opportunity to comment on drafts and approved the final version.

ETHICAL ANIMAL RESEARCH

Not applicable. The study did not involve animals.

OWNER INFORMED CONSENT

Not applicable.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13313.

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