Evaluation of an air tester for the sampling of aerosolised equine herpesvirus type 1

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EQUINE herpesvirus type 1 (EHV-1) is a major equine pathogen, responsible for well-documented syndromes of respiratory disease, abortion, neonatal foal death and myeloencephalopathy (Van Maanen 2002). Recent outbreaks at racetracks, riding schools and veterinary hospitals have provided ample documentation of the contagiousness of EHV-1 and highlight the importance of early detection (Kohn and others 2006, Henninger and others 2007). EHV-1 commonly spreads directly via horse-to-horse contact, as well as indirectly via contaminated fomites and personnel. Aerosolised droplets of respiratory secretions represent the primary route of transmission in horses with infection of the respiratory tract (Slater 2007). During recent outbreaks of neurological disease associated with EHV-1, the relative importance of direct contact and/or aerogenic transmission was not determined, due to a lack of temporal and spatial information for the affected horse populations. The titre of EHV-1 present in nasal mucus can be as high as 10^6 plaque-forming units (pfu) per swab following primary infection (Burrows and Goodridge 1972). However, viral loads in nasal secretions are lower (10^2 to 10^5 pfu/swab) in horses following either re-exposure or recrudescence of a latent state (Burrows and Goodridge 1975).

The airborne transmission of herpesviruses, such as bovine herpesvirus type 1, has been shown to occur at distances of up to 3·85 m under experimental conditions (Wentink and others 1993, Mars and others 1999). However, such information is not available for EHV-1. Air-sampling devices are used routinely in the pharmaceutical, medical and industrial settings to investigate the presence of airborne pathogens. The objective of this study was to evaluate the capability of a commercially available air tester to sample aerosolised EHV-1 for detection by real-time PCR.

The study was performed in rooms with similar air volumes of approximately 3426 m^3. The doors and windows were kept closed during each experiment. A commercially available modified life virus vaccine (Rhinomune; Pfizer Animal Health) was used for the study. The concentration of the virus, determined via real-time PCR, was 8×10^9 EHV-1 gene copies per vaccine vial (total volume 1 ml). The vaccine virus was nebulised using a commercial ultrasonic nebuliser (Devilbiss; Sunrise Medical), according to the manufacturer's guidelines, to deliver particles smaller than 5 µm. A total of four separate nebulisation events were performed, one week apart, with increasing doses of EHV-1 (8×10^6, 8×10^7, 8×10^8 and 8×10^9 gene copies). Each nebulisation event was performed in a different room.

The study protocol was as follows. The nebuliser was placed in the middle of the room, at a height of 30 cm, and 10 ml of the EHV-1 solution was nebulised over a 10-minute period, followed by an additional five minutes to allow the air to settle. Thereafter, air samples (500 litres) were collected at a height of 30 cm using a portable air-monitoring system (M Air T; Millipore) according to the manufacturer's guidelines, at 14·5, 9·6, 4·8, 1·5 and 0·5 m from the nebuliser. The portable air tester works on the basis of sampling a predetermined volume of air through a sieve directly onto a retrievable agar plate. Each sampling period lasted 3·5 minutes, and during each event, care was taken to follow a far-to-close sampling approach. The sieve of the air-sampling device was disinfected with an isopropanol wipe before the collection of each sample. Following the collection of the last sample, rayon swabs soaked in phosphate-buffered saline (PBS) were used to sample the gloves, surgical mask, coat and hair of the person performing the experiment, in order to document possible contamination. Each swab was placed in a 50 ml sterile conical tube and the handle was cut with sterile scissors. Additional air samples (1000 litres) were taken for each of the four nebulisation events from the middle of the room, one and two hours after the last sample had been taken. All the nebulisation events were performed under similar ambient temperature (29·4 to 22·4°C) and relative humidity (32 to 34 per cent). Each room was pressure-washed one day after the nebulisation event.

During the whole experiment, from the beginning of nebulisation to the collection of the last sample, 40 cm rayon swabs (Fox Converting Swabs) soaked in PBS were left in the room in a vertical position at 14·5, 9·6, 4·8, 1·5 and 0·5 m from the nebuliser, to test for environmental contamination with the EHV-1. At the end of the study, the swabs were collected, following a far-to-close approach. Each swab was placed in a sterile 50 ml conical tube and the handle was cut using sterile scissors. Cross-contamination was prevented by changing gloves between each collected swab.

The collected samples (agar plates and swabs) were processed within one hour of collection. The surface of each plate used to sample the air was washed with 1 ml PBS; the authors had previously determined that the agar had no negative effect on nucleic acid extraction or PCR amplification. For the swabs kept in conical tubes, 1 ml PBS was added to each, and each tube containing a swab was vortexed for 10 seconds, inverted and centrifuged at 16,000 *g* for five minutes; the swab was then removed from the conical tube. In order to minimise contamination, all pipetting steps were performed in a laminar flow cabinet. Nucleic acid extraction from 180 µl of plate wash or swab fluid was performed using an automated nucleic acid extraction system (CAS-1820 X-tractor Gene; Corbett Life Science) according to the manufacturer's recommendations.

All the samples were assayed for the presence of the glycoprotein B (gB) gene of EHV-1 using a previously reported real-time TaqMan PCR assay (Pusterla and others 2006). The samples were amplified in a combined thermocycler/fluorometer (7900 HTA; Applied Biosystems) with the standard thermal cycling protocol: two minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Absolute quantitation of EHV-1 target molecules was performed using a standard curve and expressed as EHV-1 gene copies per litre of sampled air or per swab.

The number of EHV-1 gene copies sampled from the air was dependent on the dose of EHV-1 nebulised and the distance at which the samples were collected (Table 1). When the two highest EHV-1 doses (8×10^9 and 8×10^8 EHV-1 gene copies) were nebulised, viral molecules were detected up to a distance of 14·5 m. The detection distance decreased to 9·6 m with the dose of 8×10^7 gene copies, and to 0·5 m with the lowest dose of 8×10^6 gene copies. One hour after collection of the last sample, EHV-1 was detected in air only with the two highest doses, at 4·6 gene copies per litre of air for the 8×10^9 dose, and 2·8 genes per litre of air for the 8×10^8 dose. Virus was not detected in any air samples collected two hours after collection of the last sample during each of the four nebulisation events. Environmental contamination, as assessed by the molecular detection of EHV-1 from swabs, was also dose and distance dependent (Table 1). Virus was detected in swabs at distances up to 14·5 m with the dose of 8×10^9 gene copies and up to 4·8 m with the dose of 8×10^8 gene copies. Contamination with EHV-1 was detected on the gloves, coat and mask following the highest dose (8×10^9) (Table 1), and

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TABLE 1: Equine herpesvirus type 1 (EHV-1) viral loads in samples of air (expressed as the number of EHV-1 glycoprotein B gene copies per litre of air) and environmental swabs (expressed as gene copies per swab) after nebulisation of different doses of EHV-1

Sample type*	EHV-1 dose (number of glycoprotein B gene copies) 8×10^9	8×10^8	8×10^7	8×10^6
Air				
At 14·5 m	60·8	7·1	–	–
At 9·6 m	154·9	33·2	0·7	–
At 4·8 m	150·7	46·4	2·54	–
At 1·5 m	145·6	46·1	1·64	–
At 0·5 m	105·1	36·0	3·21	1·72
Swab				
At 14·5 m	902	–	–	–
At 9·6 m	1605	–	–	–
At 4·8 m	2510	742	–	–
At 1·5 m	3416	2271	–	–
At 0·5 m	5328	5181	–	–
From gloves	4844	–	–	–
From coat	329	–	–	–
From mask	579	380	–	–
From hair	–	–	–	–

* Air samples and environmental swabs were sampled at preset distances from the nebuliser
– Negative real-time PCR result

on the mask following the second highest dose (8×10^8), but at the lower doses, virus was not detected in swab samples from the gloves, coat, mask or hair.

This study shows that EHV-1 can be detected by real-time PCR from air samples and environmental swabs following aerosolisation of the virus. In veterinary medicine, air sampling devices have previously been used to detect viral pathogens of chickens, such as Newcastle disease virus (Hietala and others 2005). Most applications of the air tester used in the present study are in the field of airborne microbial monitoring, and the system has been used to document the presence of *Rhodococcus equi* in air in stables and paddocks on horse breeding farms (Muscatello and others 2006). The system has no claim for the sampling of airborne viral pathogens, and the authors emphasise that these preliminary study results are not intended to replace more sensitive sampling techniques for the virus.

To the authors' knowledge, no experimental study of EHV-1 has documented the distance that viral particles can travel through the air, and it is still unknown whether EHV-1 is spread by droplet transmission (particles >5 µm) or by airborne transmission (particles <5 µm). However, on the basis of the pathophysiology of EHV-1 and the lack of explosive coughing commonly associated with clinical infections, aerosolised droplets of respiratory secretions represent the most likely primary transmission route in horses with infections of the respiratory tract. No conclusion can be drawn from the amount of virus detected in nasal secretions of horses with EHV-1 infection and the aerosolised potential of the virus. It appears, however, that the risk of infection is likely to be correlated with the viral loads in nasal secretions. The range of doses used in this study was arbitrarily chosen to represent high and low environmental loads. Due to the biohazardous nature of EHV-1 field isolates, a modified live vaccine strain was used for the evaluation of the air tester. One limitation of the study is that no conclusion can be made regarding the biological potential of the aerosolised EHV-1, since EHV-1 was detected by PCR and not culture. Cell culture would have been of limited use for evaluating the biological potential of the vaccine virus, due to the reduced replicative nature of the attenuated vaccine strain.

Assuming 100 per cent saturation of the whole room with the nebulised virus, the concentrations of the virus would have been 2·3 EHV-1 gene copies per litre of air using the lowest dose, and 2305 gene copies using the highest dose. In this study, it is likely that this situation was never achieved, since a distance-dependent viral detection was determined, and all the determined viral loads were lower than the assumed values. The length of time for which particles remain airborne and the distance they travel is unpredictable and varies mostly with the particle and organism size, the settling velocity, the nature of the fluid containing the organism, and environmental conditions such as ambient temperature, relative humidity and airflow (Garner 1996). However, the present study has shown that nebulised EHV-1 can be detected, in a dose- and distance-dependent pattern, using the commercial air tester. No evidence was found that the agar used in the air tester has an inhibitory effect on nucleic acid extraction and PCR amplification (data not shown). Additionally, in order to test whether the agar plate had a reduced efficiency for capturing EHV-1, the air coming out of the tester during each sampling time was sampled using PBS-soaked swabs. No evidence was found that nebulised EHV-1 bypasses the agar plates and is subsequently expelled via the outlet of the air tester (data not shown). Virus was not detected in any air samples collected two hours after the initial sampling session. These results support the routine practice of removing index cases from highly populated areas in order to minimise the risk of exposure to other horses.

During the study, swabs were taken from the environment and attending personnel to test for contamination by aerosolised EHV-1. Swabs placed in the environment of an affected horse for a predetermined time, or swabs collected directly from the environment of an index case, could potentially be used to determine the risk of exposure to other horses. However, this technique may not be sensitive enough to determine the true environmental contamination. The spread of EHV-1 via contaminated fomites and personnel has been implicated in several outbreaks. The results of this study highlight the importance of adhering to stringent infectious disease control strategies, using separate equipment and protective clothing to reduce indirect contact between infected and susceptible horses. Although the risk of veterinary staff inhaling aerosolised EHV-1 while managing a clinical case is rarely considered, in this study swabs collected from surgical masks tested PCR positive for EHV-1 at the two highest doses. It would have been interesting to determine whether EHV-1 can be detected in the nasal secretions of personnel, since it is known that surgical masks have poor filtration capacity and do not prevent leakage around the edge of the mask when the user inhales. It has not been determined whether EHV-1 virus inhaled by human beings can potentially infect susceptible horses.

In conclusion, these preliminary results have shown that aerosolised EHV-1 can be sampled from the air using a commercial air tester for real-time PCR detection. The air-sampling protocol used for the study will require more validation and optimisation for detection limits using naturally or experimentally infected animals in the future. The study has also shown that environmental swabs may capture aerosolised EHV-1, and that the virus may be detected from the gloves, coat and mask of attending personnel. The risks to horses of contaminated environment or personnel need to be further investigated.

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