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Journal of Virological Methods 287 (2021) 113988

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Environmental and air sampling are efficient methods for the detection and quantification of foot-and-mouth disease virus

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ARTICLE INFO

Keywords:
Environmental sampling
Surfaces
Aerosols
FMDV
Recovery efficiency
Surveillance

ABSTRACT

Foot-and-mouth disease virus (FMDV) can be found in all secretions and excretions and the breath of acutely infected animals. FMDV can survive in the environment, providing an opportunity for surveillance. The objective of this study was to assess the efficiency of sampling methods for the recovery and quantification of FMDV from a range of environmental surfaces and in aerosols. Selected surfaces, based on those likely to be found on farms, were spiked with a range of concentrations of FMDV, left to dry and then the surface was swabbed with an electrostatic dust cloth. For aerosol sampling, FMDV was nebulised at different concentrations and distances from the sampler. Recovery of viral RNA and infectious virus was measured by RT-qPCR and virus isolation respectively. FMDV RNA was detected from all surfaces at all concentrations except from glass. Infectious virus was recovered from all surfaces but only at higher concentrations. The higher the starting concentration of virus the more efficient the recovery was from surfaces and recovery was more consistent from non-porous surfaces than porous surfaces. FMDV was detected in aerosol samples and the amount of virus recovered decreased as the distance between the nebuliser and sampler increased. The higher the starting concentration of virus the more efficient the recovery was from sampled aerosols. The information provided in this study could be used to direct environmental and aerosol sampling approaches in the field and improve the detection efficiency of FMDV from an environment, thus extending the toolbox available for diagnosis and surveillance of this pathogen.

1. Introduction

For many viral diseases, the main route of transmission is by direct interactions between infectious and susceptible individuals (Keeling and Rohani, 2007; McCallum et al., 2001). However, many viruses can remain infectious outside of the host and persist on fomites in the environment for sustained periods of time under suitable environmental conditions (Boone and Gerba, 2007). The presence of virus in the environment represents an opportunity for surveillance. Environmental sampling has been utilised as a surveillance tool in the study of viruses, such as influenza virus (Indriani et al., 2010; Simmerman et al., 2010), norovirus (Stobnicka et al., 2018), poliovirus (Matsuura et al., 2000; Metcalf et al., 1995) and coronaviruses (Dowell et al., 2004).

Foot-and-mouth disease virus (FMDV) (family Picornaviridae, genus Aphthovirus) causes a highly infectious and contagious disease, foot-and-mouth disease (FMD), of wild and domesticated cloven-hoofed animals. FMDV can be shed in all secretions and excretions from acutely infected animals, including exhaled air, nasal discharge, saliva, urine and faeces, resulting in contamination of the surrounding environment (Bravo de Rueda et al., 2015; Colenutt et al., 2018, 2020). FMDV is able to persist in the environment for a prolonged period of time if the conditions are suitable (Bartley et al., 2002; Cottral, 1969), although high temperatures (>50 °C), extremities in pH (<6.0 or >9.0) and low relative humidity (<60 %) are known to cause degradation of the virus (Bachrach et al., 1957; Bøtner and Belsham, 2012; Donaldson, 1973; Turner et al., 2000). FMDV can also be detected in aerosols, providing a further opportunity for sampling the virus (Christensen et al., 2011; Nelson et al., 2017; Pacheco et al., 2017; Ryan et al., 2009).

Environmental and aerosol sampling can provide non-invasive, herd-level methods of detection to supplement surveillance for FMD. Individual sampling is not always feasible at sites where there are large numbers of animals such as live animal markets, communal grazing areas and large-scale farms. Environmental and aerosol sampling methods facilitate a less laborious sampling approach and are less stressful for animals than clinical sampling. Additionally, FMDV can be detected in the environment and in aerosols when clinical signs are not

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https://doi.org/10.1016/j.jviromet.2020.113988
Received 29 January 2020; Received in revised form 9 September 2020; Accepted 10 September 2020
Available online 7 October 2020

evident, for example in pre-clinical cases and after the recovery of animals (Colenutt et al., 2018; Nelson et al., 2017), highlighting the advantage of utilising these methods in surveillance.

In this study, we evaluated both environmental and aerosol sampling methods. The recovery of FMDV from a variety of different surfaces was assessed and data on the collection efficiency of the Coriolis micro air sampler was obtained over distances of up to 150 cm. The objective of this study is to extend the current information available on environmental and aerosol sampling methods which could help refine sampling protocols in the field. Such protocols can be used to supplement current diagnostic and surveillance techniques for improved detection of FMDV.

2. Methods

2.1. Environmental sampling

2.1.1. Virus strain

An archival virus stock of the O/UKG/34/2001 strain of FMDV, originating from the FMD outbreak in the UK in 2001, was used in this study. The virus was passaged three times in pig kidney (IB-RS-2) cells to generate sufficient material for the study. The virus titre was determined in bovine thyroid (BTY) cells as $10^{7.2}$ tissue culture infectious dose 50 ($TCID_{50}$/mL), using the Spearman-Karber method (Kärber, 1931; Spearman, 1908). 10 µl and 100 µl of the neat stock was pipetted straight onto the surfaces to spike the samples with $10^{5.2}$-$^{6.2}$ $TCID_{50}$ respectively, hereafter referred to as 10^5-10^6 $TCID_{50}$. A 10 fold serial dilution was prepared from neat stock in cell culture medium [(Glasgow minimum essential media (Gibco, Thermo Fisher Scientific, MA, USA)) with 2% antibiotics (penicillin-streptomycin, (Gibco))] to make stocks ranging from $10^{3.2}$ – $10^{6.2}$ $TCID_{50}$/mL for spiking materials. From these dilutions, a 10 µl volume was used for spiking materials with $10^{1.2}$ – $10^{4.2}$ $TCID_{50}$, hereafter referred to as 10^1-10^4 $TCID_{50}$.

2.1.2. Cloth inactivation

A preliminary study was carried out to assess if the electrostatic dust cloth swabs (Minky Homecare, Rochdale, UK) used for sample collection inactivated FMDV. 100 µl and 10 µl of the neat stock was spotted straight onto the cloth to spike the sample with 10^{5}-^{6} $TCID_{50}$ respectively. 10 µl of 10^3-10^6 $TCID_{50}$/mL concentration of virus was spotted straight onto the cloth to spike the sample with 10^{1}-^{4} $TCID_{50}$ respectively. Each cloth was spiked in a class II microbiological safety cabinet (MBSC) and processed according to the methods described in Section 2.1.4.

2.1.3. Spiking experiments

A series of experiments were performed to assess recovery of FMDV from the surfaces of materials selected based on those likely to come into contact with livestock on a farm. Specifically, the materials tested were: planed softwood (Homebase, Milton Keynes, UK) cut into approximately 2 cm^3 blocks; 8 mm thick cotton rope cut into 8 cm segments; red garden bricks broken into approximately 2 cm^3 blocks; 2.5cm × 1.5cm steel metal discs (F.H.Brundle, Southampton, UK); 8.5 cm^2 squares of bonded plastic from a laboratory tray; and 7.6 × 2.6 cm glass slides (Consumable Solutions Ltd, Portsmouth, UK).

Inside a class II MBSC, each surface material (wood, rope, brick, steel, plastic and glass) was spiked with a range of virus doses (10^1-10^6 $TCID_{50}$/mL); 100 µl and 10 µl of the neat stock was spotted straight onto the surface to spike the sample with 10^{5}-^{6} $TCID_{50}$ respectively. 10 µl of 10^3-10^6 $TCID_{50}$/mL concentration of virus was spotted straight onto the surface to spike the sample with 10^{1}-^{4} $TCID_{50}$ respectively. The virus was administered via pipette in small droplets and spread over the surface with a sterile pipette tip. The virus was left to dry on the surface for approximately 30 min before the surface was swabbed with an electrostatic dust cloth.

The inoculum used to spike materials was also added directly into 10 ml of laboratory medium and 5 ml of field medium and processed by each method to provide a control for recovery from these samples. Two control samples were taken from each concentration of virus.

2.1.4. Swab processing methods

Two methods were used for processing swabs. The first was developed during environmental sampling in a laboratory environment (Colenutt et al., 2020). The second was adapted from the first for use in the field (Colenutt et al., 2018), where it was not feasible to use the equipment required for the laboratory method.

Laboratory method: Electrostatic dust cloths were cut into 15 × 10.5 cm swabs and were either spiked (cloth inactivation experiment) or were used to swab a surface contaminated with a known amount of virus (spiking experiment). The dust cloth was then placed in 10 ml of cell culture medium in a 50 ml conical centrifuge tube (Thermo Fisher Scientific). The cloth swabs were fully saturated in medium, then agitated using a mains-powered mechanical vortex for approximately 10 s to elute the virus from the cloths. A disposable wooden spatula (VWR, PA, USA) was used to remove the swab, at the same time pressing it to the wall of the tube to extract as much medium as possible.

Field method: Electrostatic dust cloths, cut into 7 × 11 cm swabs, were spiked or used as swabs as described for the laboratory method samples and placed in 5 ml impinger fluid [Glasgow minimum essential media (Gibco) with antibiotics (penicillin-streptomycin and amphotericin-B (Gibco)), 5% BSA (Sigma-Aldrich, MO, USA) and 1 M HEPES (Gibco)] in a screw top pot (VWR). The pot containing the swab and medium was manually shaken to fully saturate the cloth. A disposable wooden spatula (VWR) was used to remove the swab, at the same time pressing it to the wall of the pot to extract as much medium as possible.

Samples for both the cloth inactivation and spiking experiments were carried out in triplicate for each processing method and for every material sampled. For both methods aliquots were taken from the medium and stored at −80 °C prior to laboratory analysis. To reduce operator bias (due to, e.g. a difference in applied pressure), the same person (EB) carried out all the experiments.

2.1.5. Laboratory analysis

All samples were tested by reverse transcription quantitative PCR (RT-qPCR). Viral RNA was extracted from samples using the KingFisher Flex automated extraction platform (Thermo Fisher Scientific) with the MagMAX™-96 Viral RNA Isolation kit (Thermo Fisher Scientific). Specifically, 50 µl of the cloth eluate was used in the extraction and eluted into a final volume of 90 µl. FMD viral RNA was detected by RT- qPCR on the ABI 7500 system (Applied Biosystems, Thermo Fisher Scientific) using the Callahan assay to target the 3D region of the FMDV genome (Callahan et al., 2002). A 10^6 $TCID_{50}$/mL suspension of the same virus stock used in the experiments was prepared and a 10-fold dilution series of the suspension was used as a standard curve for virus load quantification. The standard curves were extracted in the same manner as described for the samples and run on the same extraction and PCR plate. The samples were quantified in $TCID_{50}$/mL from the standard curves.

All samples were tested for the presence of infectious virus by virus isolation (VI). Briefly, virus concentration was quantified by inoculating 10-fold serial dilutions of samples and inoculating 200 µl of each sample onto monolayers of bovine thyroid (BTY) cells as described previously (Snowdon, 1966).

2.2. Aerosol sampling

2.2.1. Virus strains

Three strains of FMDV, kept as archival virus stocks at The Pirbright Institute, UK, were used in the study: O/UKG/34/2001, A/TAI/17/2016, and ASIA1/SHAMIR/VV/2001 (hereafter denoted O, A and Asia 1, respectively). These were selected to represent the most widely distributed serotypes of FMDV. The viruses were passaged, once on BTY cells and three times in swine renal (IB-RS-2) cells, to generate sufficient material for the experiment. Three virus stocks at concentrations of 10^2, 10^4 and 10^6 $TCID_{50}$/mL were made from the passaged stock.

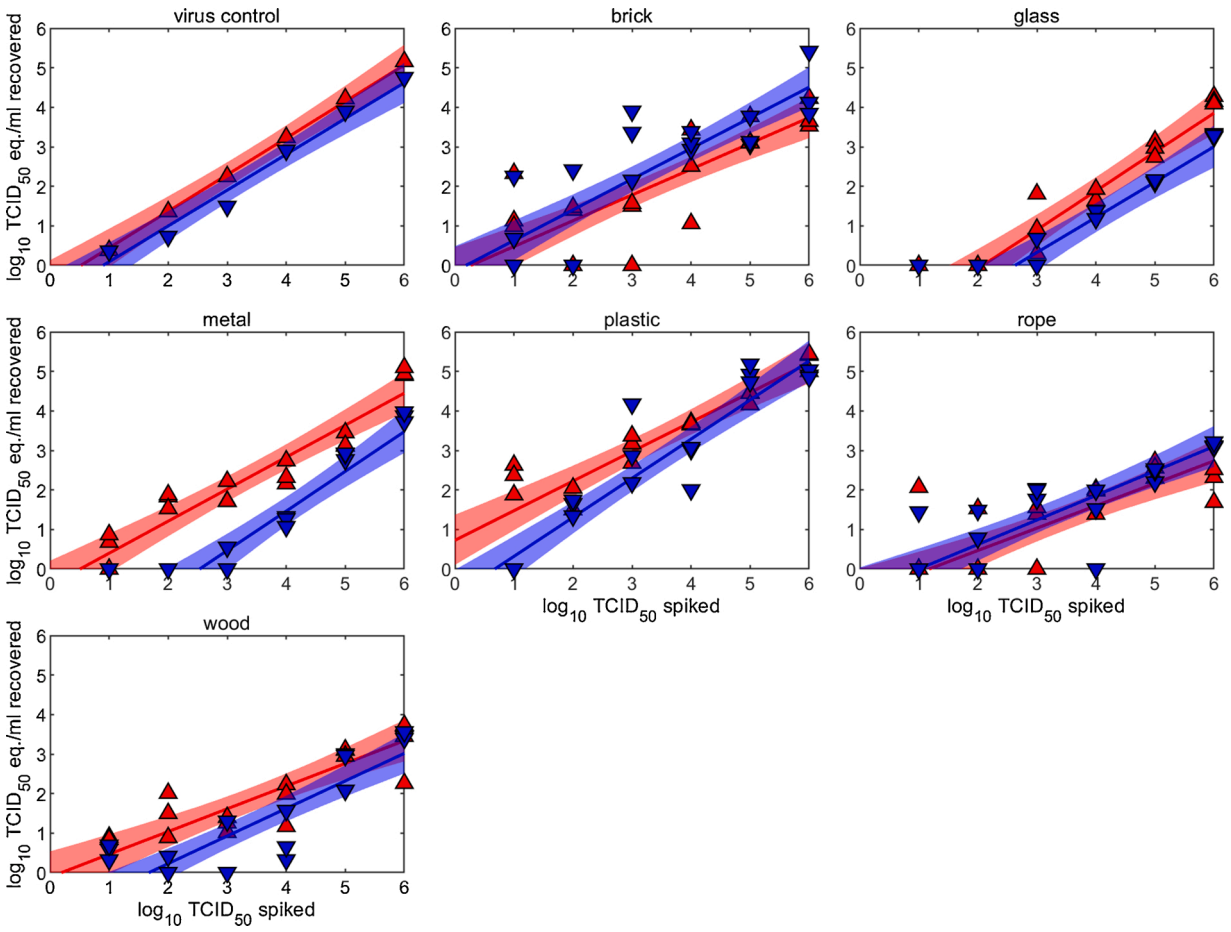

Fig. 1. Recovery of foot-and-mouth disease viral RNA from different surfaces contaminated with the virus. Symbols show the amount of viral RNA (log_{10} $TCID_{50}$ equivalents/mL) recovered from the surface (indicated above the plot) when swabbed and processed using the field (red) or laboratory (blue) method after spiking with different titres of virus (log_{10} $TCID_{50}$). The lines and shading show the posterior median and 95 % credible interval for the expected amount of viral RNA recovered, respectively (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

2.2.2. Generating and collecting aerosols

The study was carried out in an isolation room, operated under negative pressure, within the high containment isolation facilities at The Pirbright Institute, UK. To avoid interference from the air handling processes a 2.7 m clear plastic tunnel (Stadia Sports, Newcastle) was used to house the air sampler and nebuliser. The temperature and relative humidity were maintained throughout the study at 19 °C and 50–60 % RH, respectively.

The OMRON CompAir NE-C28 Compressor Nebulizer (OMRON Healthcare, Milton Keynes, UK) was used as per the manufacturer's instructions to generate virus aerosols. The Coriolis micro air sampler (Bertin Technologies, Aix-en-Provence, France) is a wet-walled cyclone sampler which functions as an impinger device. The Coriolis micro air sampler was run for 10 min at a flow rate of 300 L/min and collected aerosolised material into impinger fluid. Aerosol samples were collected at 10 cm, 75 cm and 150 cm from the nebuliser, in triplicate for each distance. Each run consisted of running the sampler for one minute, after which the nebuliser was switched on and run for three minutes allowing 1 ml of virus suspension to be aerosolised. The nebuliser was then switched off enabling collection of any residual aerosolised virus by the air sampler for a further six minutes. Figure S.1 shows the set-up of the air sampler and nebulizer within the plastic tunnel. An aliquot of the aerosolised stock was collected and used as a control for laboratory assays.

2.2.3. Disinfection and deposited aerosols

After each sample collection the inside of the tunnel, the air sampler and the nebuliser were disinfected with 0.2 % citric acid (Sigma-Aldrich), rinsed with water and dried with a paper towel to avoid cross contamination between runs. To further minimise the potential for cross contamination one viral strain was used per day, with experiments carried out sequentially over three days. At the end of sampling each day, swabs were collected to ensure cleaning protocols were effective: one from the area immediately around the nebuliser, one from the middle of the tunnel and one from the area immediately around the air sampler, pre and post implementation of the cleaning protocol. All swabs taken from the tunnel were negative by RT-qPCR which confirmed the cleaning protocols were effective (data not shown). Additionally, there was no cross contamination observed between the experiments, as demonstrated by a serotype specific PCR (data not shown). At the end of sampling each day the equipment and room surroundings were thoroughly disinfected with 0.2 % citric acid (Sigma-Aldrich).

2.2.4. Laboratory processing and analysis

Following the end of sampling each day, aerosol samples were transferred to the laboratory at The Pirbright Institute, UK for processing. Aliquots of the impinger fluid were made and stored at −80 °C until laboratory analysis could take place. All samples were tested by RT-qPCR and VI as previously described in Section 2.1.5.

2.3. Statistical methods

The effect of surface type and sample processing method on recovery

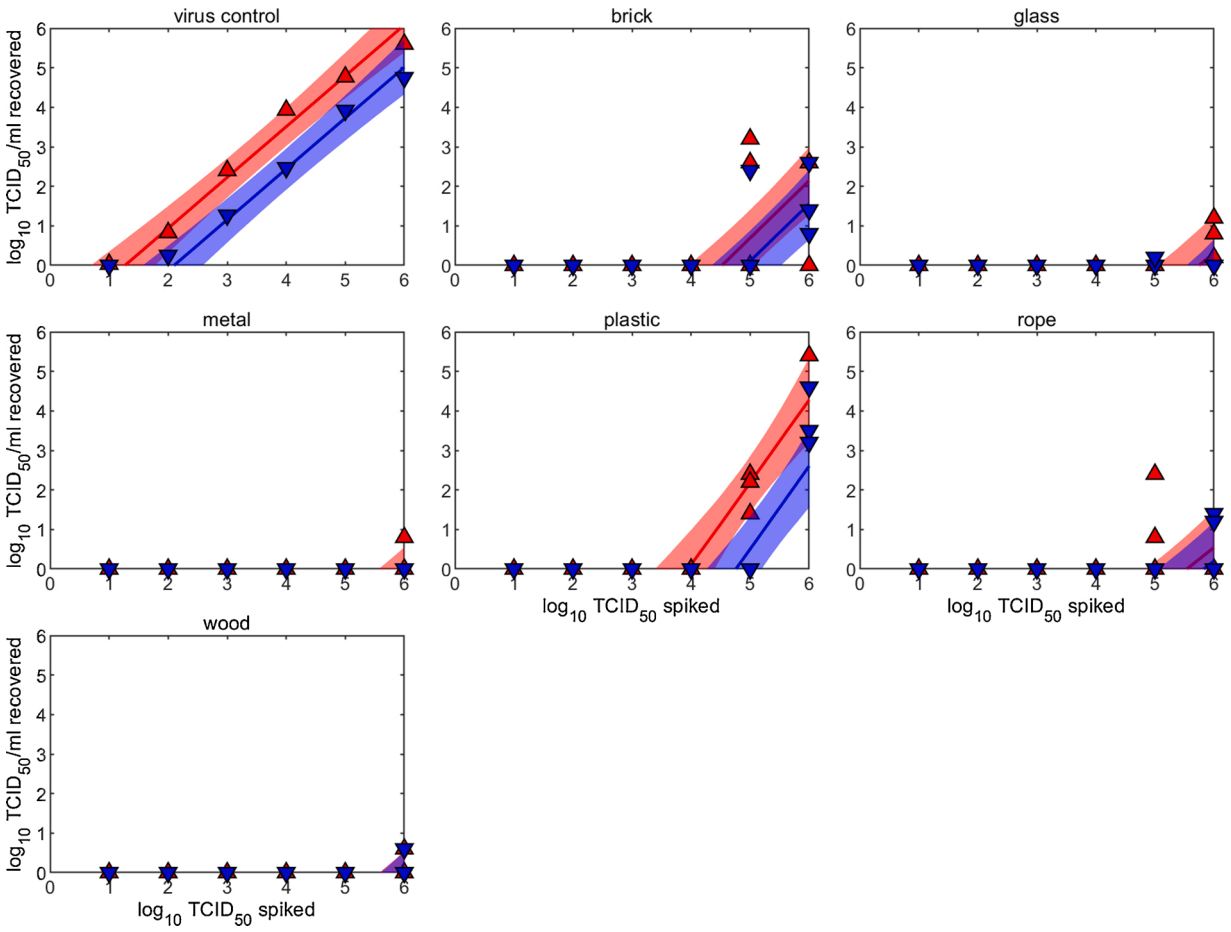

Fig. 2. Recovery of foot-and-mouth disease virus from different surfaces contaminated with the virus. Symbols show the amount of infectious virus (log_{10} $TCID_{50}$/mL) recovered from the surface (indicated above the plot) when swabbed and processed using the field (red) or laboratory (blue) method after spiking with different titres of virus (log_{10} $TCID_{50}$). The lines and shading show the posterior median and 95 % credible interval for the expected amount of infectious virus recovered, respectively (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

of viral RNA or infectious virus was assessed using Bayesian hierarchical models (see Text S1 for full details). In this approach, straight lines were fitted to data on the amount of viral RNA or infectious virus recovered at each spiking titre. The slopes and intercepts of the lines were allowed to vary amongst surface types (virus control, brick, glass, metal, plastic, rope or wood) and processing methods (field or laboratory) by assuming they are drawn from higher-order hierarchical distributions. A range of models were considered for the slopes and intercepts, in which they were either independent of surface type and processing method, depended on one of them or depended on both of them (see Table S1). The different models were compared using the deviance information criterion (DIC) (Spiegelhalter et al., 2002). The methods used to estimate the slopes and intercepts allowed for a detection threshold at 1 $TCID_{50}$ (equivalent)/mL by left-censoring observations at (or below) this threshold. Using this detection threshold, the limit of detection for each surface and method was computed from the fitted lines as -intercept/slope.

The air sampling data were analysed in a similar way, except the slopes and intercepts in the models depended on virus strain (O, A or Asia 1) and distance from the nebuliser (10, 75 or 150 cm) (see Table S2 for the different models considered for the slopes and intercepts).

3. Results

3.1. Inactivation of virus on electrostatic dust cloths

When FMDV was spiked directly onto the electrostatic dust cloths, viral RNA was detected in samples from all doses when processed by the field method and down to 10^2 $TCID_{50}$ in samples processed by the laboratory method (Figure S.2). Infectious FMDV was recovered from the cloths when samples were spiked with 10^3 $TCID_{50}$ or higher for both methods (Figure S.3). There was a $10^{0.5}$-10^1 fold reduction in titre of FMDV RNA and infectious virus for cloth samples spiked with higher amounts of virus (10^5 $TCID_{50}$ and 10^6 $TCID_{50}$). However, recovery of FMDV RNA was less efficient at the lower amounts of virus.

3.1.1. Recovery of virus from environmental surfaces

FMDV RNA was detected in samples taken from plastic, metal, wood, brick and rope when spiked with doses from 10^1 $TCID_{50}$ to 10^6 $TCID_{50}$ (Fig. 1). By contrast, viral RNA was not recovered from glass at concentrations of 10^1 $TCID_{50}$ or 10^2 $TCID_{50}$ (Fig. 1). Recovery of viral RNA was most efficient from plastic with a 10°-10^2 fold reduction in recovered titre compared to the spiked titre at all concentrations and processing methods. Recovery of viral RNA was least efficient from rope, with a 10^3-10^4 fold reduction in recovered titre compared to the spiked titre for samples spiked with doses of virus between 10^4 and10^6 $TCID_{50}$ (Fig. 1). Generally, the recovery rates of FMD viral RNA were comparable between the two processing methods.

Infectious FMDV was recovered using both processing methods from all surfaces only when spiked with 10^5 or 10^6 $TCID_{50}$ (Fig. 2). In general, higher levels of infectious FMDV were detected using the field processing method compared to the laboratory method.

When fitting straight lines to the levels of viral RNA recovered (Fig. 1) or the levels of infectious virus recovered (Fig. 2), changes in the

Table 1
Limits of detection (posterior median (95 % credible interval)) for foot-and-mouth disease virus RNA and infectious virus in environmental swabs taken from different surfaces and processed using two methods.

Surface	Method	Viral RNA (log_{10} $TCID_{50}$ equivalents/mL)	Infectious virus (log_{10} $TCID_{50}$/mL)
Brick	Laboratory	0.2 (0, 0.9)	4.9 (4.4, 5.5)
	Field	0.3 (0, 1.0)	4.5 (3.9, 5.1)
Glass	Laboratory	2.6 (2.0, 3.2)	6.4 (5.5, 7.9)
	Field	2.1 (1.5, 2.6)	5.7 (5.0, 6.7)
Metal	Laboratory	2.5 (2.0, 3.0)	≥6‡
	Field	0.5 (0, 1.1)	≥5.6‡
Plastic	Laboratory	0.7 (0.03, 1.2)	4.8 (4.3, 5.2)
	Field	0†	3.9 (3.4, 4.4)
Rope	Laboratory	1.0 (0, 1.7)	5.8 (5.1, 6.9)
	Field	1.2 (0, 1.9)	5.5 (4.8, 6.5)
Wood	Laboratory	1.7 (1.0, 2.2)	≥5.6‡
	Field	0.2 (0, 1.1)	≥5.6‡

† Posterior median and 95 % credible limits all below detection threshold at 1 $TCID_{50}$ eq./mL.
‡ Posterior median and upper 95 % credible limit >6 log_{10} $TCID_{50}$/mL.

DIC for different models showed that both intercepts and slopes of the lines differed significantly between the surface types and the processing method used (Table S.1).

3.1.2. *Limits of detection from environmental surfaces*

The detection limits (i.e. the spiked titre at which the recovered titre is zero) were calculated for each surface tested. The lowest detection limits were from brick when processed by the laboratory method (0.2 log_{10} $TCID_{50}$ equivalents/mL) and from plastic when processed by the field method (0 log_{10} $TCID_{50}$ equivalents/mL) (Table 1). The highest detection limits were from glass when processed by the laboratory and field methods (2.6 log_{10} $TCID_{50}$ equivalents/mL and 2.1 log_{10} $TCID_{50}$ equivalents/mL, respectively). Generally, detection limits were comparable for samples processed by the field method compared to the laboratory method (Table 1).

The limit for detecting infectious virus was generally higher than detection of viral RNA (Table 1& 2). The lowest limit of infectious virus detection was from plastic when processed by the laboratory and field methods, with a detection limit of 4.8 log_{10} $TCID_{50}$/mL and 3.9 log_{10} $TCID_{50}$/mL respectively. The highest limit of detection for infectious virus was from metal and wood when processed by both methods, with a detection limit of >6 log_{10} $TCID_{50}$/mL. Generally, the limit of detection for infectious virus was lower for samples processed by the field method than the laboratory method.

3.1.3. *Recovery of virus from aerosol samples*

Three strains of FMDV (denoted O, A and Asia 1) were nebulised at three concentrations to test the efficiency of the Coriolis in detecting FMDV aerosols at varying distances. The results show that all strains were detectable in aerosol samples. Recovery of viral RNA and infectious virus was most efficient from strain A than the other two strains. Viral RNA and infectious virus were detected at all concentrations from strain A and at concentrations of 10^6 $TCID_{50}$/mL and 10^4 $TCID_{50}$/mL for strains O and Asia 1 (Figure 3 &4). The higher the starting concentration of nebulised virus the more efficient the recovery was from sampled

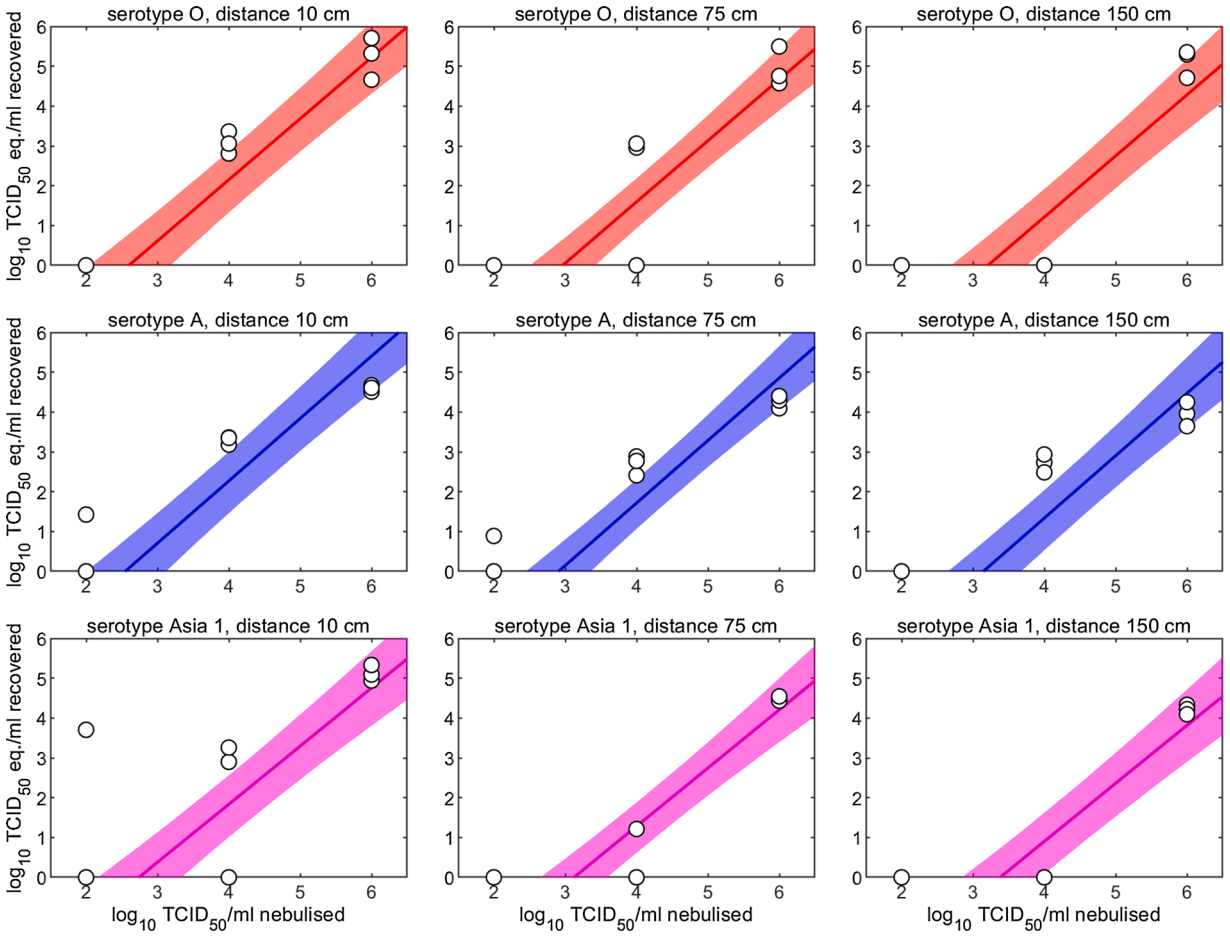

Fig. 3. Recovery of foot-and-mouth disease viral RNA in air samples collected at different distance from a nebuliser. Circles show the amount of viral RNA (log_{10} $TCID_{50}$ equivalents/mL) recovered in the air sample at each distance for each strain (indicated above the plot) when different titres of virus were nebulised (log_{10} $TCID_{50}$/mL). The lines and shading show the posterior median and 95 % credible interval for the expected amount of viral RNA recovered, respectively.

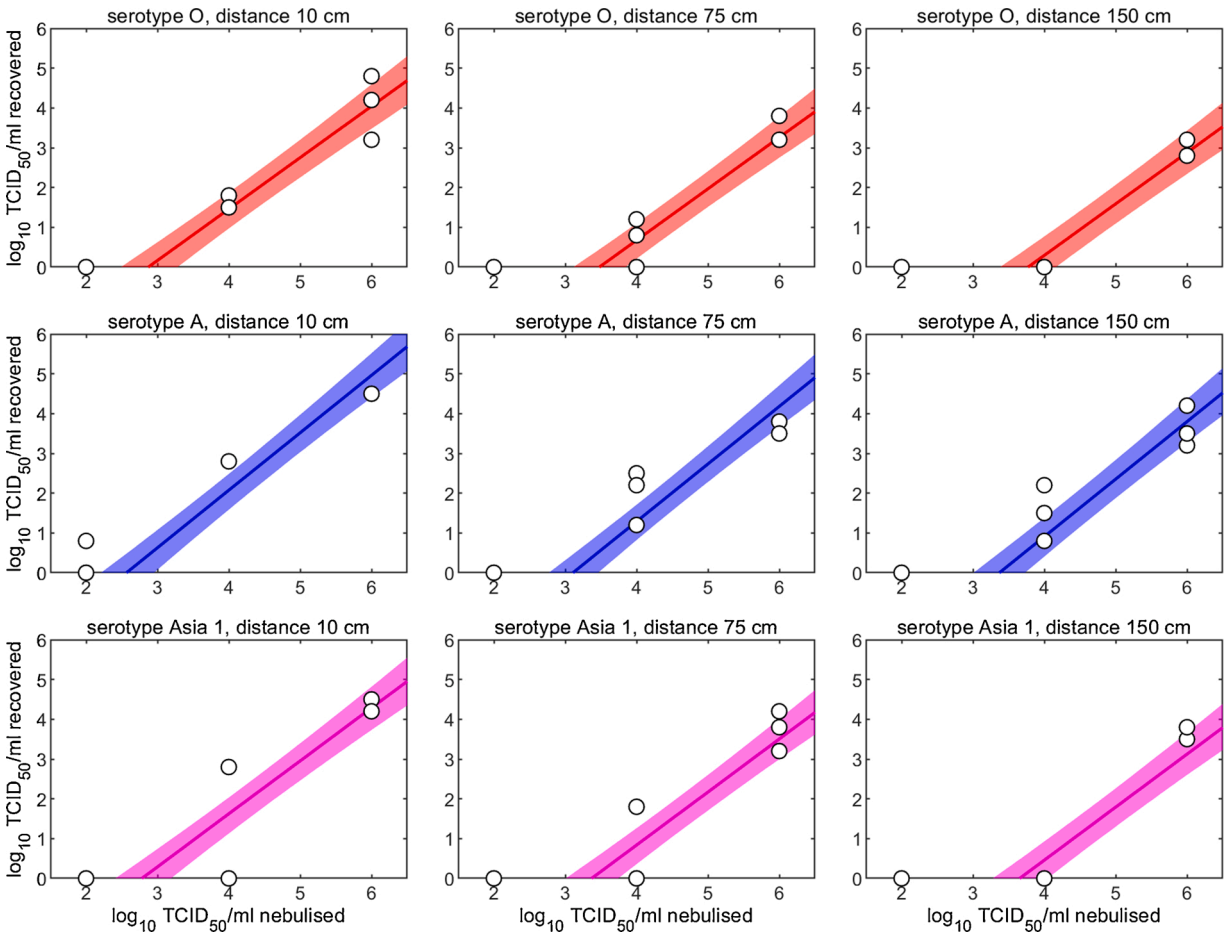

Fig. 4. Recovery of foot-and-mouth disease virus in air samples collected at different distance from a nebuliser. Circles show the amount of infectious virus (log_{10} $TCID_{50}$/mL) recovered in the air sample at each distance and for each strain (indicated above the plot) when different titres of virus were nebulised (log_{10} $TCID_{50}$/mL). The lines and shading show the posterior median and 95 % credible interval for the expected amount of infectious virus recovered, respectively.

Table 2
Limits of detection (posterior median (95 % credible interval)) for foot-and-mouth disease virus RNA and infectious virus in air samples for three strains when collected at different distances from a nebulizer.

Strain	Distance (cm)	Viral RNA (log_{10} $TCID_{50}$ equivalents/mL)	Infectious virus (log_{10} $TCID_{50}$/mL)
O	10	2.6 (2.0, 3.2)	2.9 (2.5, 3.3)
	75	3.0 (2.5, 3.4)	3.5 (3.1, 3.8)
	150	3.2 (2.7, 3.8)	3.8 (3.4, 4.2)
A	10	2.5 (2.0, 3.1)	2.6 (2.2, 3.0)
	75	2.9 (2.5, 3.4)	3.1 (2.8, 3.5)
	150	3.1 (2.6, 3.7)	3.4 (3.0, 3.7)
Asia 1	10	2.7 (2.2, 3.3)	2.8 (2.4, 3.2)
	75	3.1 (2.7, 3.6)	3.4 (3.0, 3.7)
	150	3.4 (2.8, 3.9)	3.7 (3.3, 4.0)

aerosols. The further the air sampler was from the nebuliser the less viral RNA or infectious virus was recovered.

Straight lines were fitted to the levels of viral RNA recovered (Fig. 3) and the levels of infectious virus recovered (Fig. 4). Comparing different models using the DIC showed that the intercepts of the lines differed significantly with distance while the slopes differed significantly with viral strain (Table S.2).

3.1.4. Limits of detection from aerosol samples

The detection limits for aerosolised FMDV were calculated for each strain and distance tested (Table 2). In all instances the limit of detection increased as the distance from the nebuliser to the air sampler increased. The lowest detection limit for FMDV RNA and infectious virus at all distances was strain A (Table 2). The highest limit of detection for viral RNA and infectious virus at each distance was strain Asia 1 and strain O, respectively (Table 2).

4. Discussion

When outbreaks occur, FMDV is shed in high quantities in secretions and excretions from acutely infected animals and disseminates rapidly and effectively into the environment where it can survive for long periods of time under favourable conditions. Therefore, sampling from the environment around infected animals can provide a useful tool for FMD surveillance. In this paper, we validated the use of electrostatic dust cloths by assessing the efficiency with which they recover FMDV from specific surfaces. Recovery of viral RNA and infectious virus was quantified for a range of materials, selected based on those likely to be found on farms.

Our results show that viral RNA was recovered from all surfaces tested, using both laboratory and field processing methods. Recovery was more efficient at higher inoculum levels and was more consistent from non-porous surfaces (plastic, metal and glass) than porous surfaces (wood, rope and brick) (Fig. 1). It is likely that the physical properties of a surface could affect the recovery of virus (Scherer et al., 2009; Taku et al., 2002; Turnage and Gibson, 2017). For example, it could be hypothesised that recovery of virus would be more efficient from non-porous surfaces than porous as virus could be entrapped in the crevices of porous surfaces causing incomplete removal of virus from the surface. Whereas, non-porous surfaces such as plastic or metal are

smooth which could increase the efficiency of swabbing as virus particles are easier to remove from the surface (Scherer et al., 2009). However, based on the data presented in this paper we were not able to make any firm conclusions as to whether porous or non-porous surfaces were more efficient at recovering FMDV.

We compared two methods for processing environmental swabs, one developed for use in the laboratory and the other adapted for use in the field. Both the laboratory and field methods had a comparable efficiency for recovering FMDV from surfaces, demonstrating the efficiency of the sampling method is not reliant on the use of laboratory equipment. Recovery of virus using the field method was aided by reducing the volume of medium from 10 ml to 5 ml.

During the pre-clinical and acute phases of disease, FMDV is disseminated in secretions and excretions of infected animals to the surrounding environment. The virus is usually cleared by an animal at 10–14 days post-infection and is no longer detectable in their secretions and excretions (Alexandersen et al., 2003). However, the virus can persist for weeks or months in the environment if the conditions are favourable, albeit at low levels (Bartley et al., 2002; Colenutt et al., 2018; Cottral, 1969). Therefore, it is important that the environmental sampling method used is sensitive enough to detect low levels of virus or viral RNA. The results of this study show recovery was more efficient at higher inoculum levels, indicating a decreased sensitivity of the swab method if the contamination level is low. However, viral RNA was still recovered from surfaces when spiked with low doses of virus.

In previous research, a range of aerosol sampling devices have been used to measure FMDV aerosols. However, many of these devices are unsuitable for field use as they require a vacuum pump, typically run from a mains power supply and smaller devices which have been previously used are no longer commercially available (Ryan et al., 2009). Additionally, some devices are not easily decontaminated in the field, making them less suitable for purpose. In this study we measured the efficiency of the Coriolis micro air sampler to detect FMDV aerosols at three distances for three strains of FMDV. The result shows recovery was significantly higher and limit of detection lower from the strain A virus than strains O and Asia 1. This finding supports previous research which suggests generally serotype A viruses are more stable than other serotypes of FMD. Donaldson (1973) found that serotype A viruses were more stable in aerosols than serotype O and C when exposed to 55 % and 70 % relative humidity. Scott et al. (2019) demonstrated that the serotype A virus A24 Cruzeiro was generally more thermostable than serotype SAT 1, 2 and 3 viruses.

In this study, nebulised virus with a titre of 10^2 $TCID_{50}$/mL was not detected by the Coriolis air sampler for strains of serotype O and Asia 1 and only detected at 10 cm from the nebuliser for strain A. The results suggest that this sampler may not be sensitive enough to detect a few infected individuals in a herd-level situation. However, a nebuliser was used as the source of aerosols and, therefore, it may be difficult to extrapolate to a real life situation where emissions from acutely infected animals are the source of virus. Further work could expand on this research by investigating the use of the Coriolis in measuring emissions from infected animals in situations where a large number of animals are present.

Due to biosecurity restrictions associated with aerosolising FMDV the aerosol experiments were performed once, however the authors acknowledge that there may be day-to-day variation and a larger data set, with data collected on different days, would strengthen our conclusions.

This study demonstrated that the environmental and air sampling methods presented here are suitable for detecting FMDV RNA, thus extending the toolbox available for diagnosis and surveillance of this pathogen. However, optimisation of these methods for recovery of infectious virus is needed.

In addition, further applications of these methods could be explored. For example, the environmental and air sampling methods described in this study could be investigated for use in detecting other pathogens known to survive in the environment for long periods of time, such as lumpy skin disease virus (Abera et al., 2015) and African swine fever virus (Gogin et al., 2013).

Funding

This work was funded by the Department for Environment, Food and Rural Affairs (Defra) (grant code: SE2816).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Emma Brown: Methodology, Validation, Investigation, Writing - original draft, Visualization. **Noel Nelson:** Methodology, Investigation, Writing - review & editing. **Simon Gubbins:** Formal analysis, Writing - review & editing. **Claire Colenutt:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision.

Acknowledgements

The authors are grateful to the Animal Service department at The Pirbright Institute for their help with the set-up of the aerosol sampling experiments.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2020.113988.

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