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Quantification of airborne African swine fever virus after experimental infection

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ABSTRACT

Knowledge on African Swine Fever (ASF) transmission routes can be useful when designing control measures against the spread of ASF virus (ASFV). Few studies have focused on the airborne transmission route, and until now no data has been available on quantities of ASF virus (ASFV) in the air. Our aim was to validate an air sampling technique for ASF virus (ASFV) that could be used to detect and quantify virus excreted in the air after experimental infection of pigs. In an animal experiment with the Brazil'78, the Malta'78 and Netherlands'86 isolates, air samples were collected at several time points. For validation of the air sampling technique, ASFV was aerosolised in an isolator, and air samples were obtained using the MD8 air scan device, which was shown to be suitable to detect ASFV. The half-life of ASFV in the air was on average 19 min when analysed by PCR, and on average 14 min when analysed by virus titration. In rooms with infected pigs, viral DNA with titres up to $10^{3.2}$ median tissue culture infective dose equivalents ($TCID_{50}$ eq.)/m^3 could be detected in air samples from day 4 post-inoculation (dpi 4) until the end of the experiments, at dpi 70. In conclusion, this study shows that pigs infected with ASFV will excrete virus in the air, particularly during acute disease. This study provides the first available parameters to model airborne transmission of ASFV.

1. Introduction

African swine fever virus (ASFV) is the causative agent of African swine fever (ASF), a notifiable swine disease (OIE listed disease). Since there is no vaccine available for ASF, the main strategies for control during outbreaks are animal stamping out, movement restrictions and increased hygiene. These control measures lead to a considerable economic impact (Costard et al., 2009). Development of control strategies with less economic impact requires further insights into the epidemiology of ASF and into its transmission routes.

Epidemiological studies investigated several transmission routes for ASFV (Plowright et al., 1994). In addition to being tick borne (Plowright et al., 1994), ASFV can be transmitted either by direct contact, between pigs and between wild boar; or by indirect contact, through ASFV contaminated fomites or swill feeding (Costard et al., 2009). Transmission involves virus shedding by infected pigs in all excretions and secretions, with particularly high levels in oral-nasal fluid (de Carvalho Ferreira et al., 2012; Ekue et al., 1989; Greig and Plowright, 1970; Wilkinson et al., 1983) and occasionally in faeces (de Carvalho Ferreira et al., 2012; McVicar, 1984). These infectious secretions and excretions may become aerosolised

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through sneezing, coughing or splattering of faeces and urine. When dried, secretions and excretions can be present in dust, which can also become aerosolised. In both situations, ASFV may be available in the air and may potentially lead to airborne ASFV transmission.

So far, only a few studies on ASFV airborne transmission have been published. The first attempt to demonstrate ASFV airborne transmission under experimental conditions, between pigs separated by double-wire fencing, was not successful (Montgomery, 1921). Wilkinson (1977), on the other hand, was able to demonstrate airborne transmission of ASFV after continuously exposing susceptible pigs to air from infected pigs, both through an air duct and by keeping the contact pigs on a platform 2.3 m above the infected pigs for 48 h. However, in both experiments no transmission occurred to adjacent pens at distances of more than 2.3 m (Wilkinson et al., 1977) and ASFV was not detected in the air (Wilkinson et al., 1977). These observations led researchers to assume that the airborne spread of ASFV might only be a problem within intensive housing systems (Wilkinson et al., 1977). However, no further studies have been published addressing the issue of airborne transmission or the quantification of virus transmission within intensive housing systems. Modelling the probability of airborne transmission of ASF requires data on the quantity of airborne ASFV excreted by infected pigs, the minimum infectious dose and the survival of ASFV in the air (Stark, 1999). For ASFV, knowledge on these parameters is still lacking.

In order to detect and quantify the amount of airborne ASFV, air samples were taken during infection experiments with three different ASFV isolates. The sampling method was validated and survival of ASFV in the air was assessed under *in vitro* conditions.

2. Materials and methods

2.1. Virus

Three isolates were used in our study. The Netherlands'86 and the Malta'78 are considered to be moderately virulent. The Brazil'78 is considered to be highly virulent, based on the observation that inoculated animals died within nine days after inoculation (de Carvalho Ferreira et al., 2012).

2.2. Air sampling method

Samples were taken using the MD8 air scan sampling device (Sartorius, Nieuwegein, The Netherlands) and sterile gelatine filters of 3 μm pore size and 80 mm diameter (type 17528-80-ACD; Sartorius). Two air sampling protocols were used: an air speed of 2 m^3/h for 2 min and an air speed of 8 m^3/h for 10 min. More details about the sampling method are described in Weesendorp et al. (2008).

2.3. Gelatine filter effect on ASFV detection and stability

Samples were taken to test whether gelatine filters used for air sampling had a negative effect on detection and stability of ASFV. A total of 36 filters were inoculated with 0.5 ml of approximately 10^5 median tissue culture infective dose ($TCID_{50}$)/ml of the Brazil'78, Malta'78 or Netherlands'86 isolates. For each ASF isolate under study, three different volumes of medium (5, 10 and 25 ml) were used to dissolve the filters. The filters were dissolved at two different time points after inoculation (5 min and 30 min), to mimic a delay in the time between sampling and processing of samples. Every experiment was done in duplicate for all combinations. Filters were dissolved in Eagle minimum essential medium (EMEM) (Gibco, Invitrogen, Breda, The Netherlands), supplemented with 5% foetal bovine serum and 10% antibiotics; kept at 37 °C. Immediately after being dissolved, the samples were stored at −70 °C. For each sample, matching virus stock controls were taken. These controls were exposed to exactly the same conditions regarding volume of medium and processing time, except for the gelatine filter inoculation step. All the samples were tested by quantitative real-time polymerase chain reaction (qPCR) and virus titration (VT).

2.4. Experimental aerosol production

Brazil'78, Malta'78 and Netherlands'86 isolates, with titres of approximately 10^5 $TCID_{50}$/ml, were used for experimental aerosol production. Per ASFV isolate, 10 ml were aerosolised in an empty isolator with a volume of 0.87 m^3 (1.45 m × 0.66 m × 0.91 m), as described previously by Weesendorp et al. (2008).

Between each aerosol experiment, the ventilation (81.5 m^3/h) was switched on for 15 min and control air samples were taken to confirm that ASFV concentrations in the isolator were below the detection limit after ventilation.

2.5. Air sampling procedure to determine ASFV half-life in the air

Samples were taken using a sampling speed of 2 m^3/h for 2 min; thus sampling 67 litres of air. The air inside the isolator was sampled at the following times: $t = 0$ (at the end of aerosol production), $t = 15$ min, $t = 30$ min and $t = 45$ min. The gelatine filters were then transported to the lab within 30 min after sampling, and dissolved in 10 ml of medium, kept at 37 °C for 5 min. All the samples were tested in qPCR and VT. In order to calculate the half-life of ASFV in the aerosols, a linear regression model was used to fit the observed data. Using the regression equation:

$$E = \text{slope} \times t + C$$

E = virus concentration after 15, 30 or 45 min ($t = 15$, $t = 30$ or $t = 45$), with t = time interval in min, C = virus concentration at the start ($t = 0$). The half-life ($t_{1/2}$) of ASFV in the aerosols was considered to be the time when the sampled filters titres decreased 50% or $\log_{10}$ (0.5) $TCID_{50}$. The equation above can then be rearranged: $t_{1/2} = \frac{\log_{10}(0.5)}{\text{slope}}$

2.6. Sampling procedure to determine ASFV sedimentation rate

In a closed system, it can be assumed that ASFV removed from the air will sediment on surfaces (Morawska, 2006). In order to characterise the sedimentation of airborne ASFV on surfaces and compare it to the half-life of