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Aerosol transmission of foot-and-mouth disease virus Asia-1 under experimental conditions

C. Colenutt[a,*], J.L. Gonzales[a,c], D.J. Paton[a], J. Gloster[a,b], N. Nelson[a,b], C. Sanders[a]

[a] The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 0NF, UK
[b] Met Office, FitzRoy Rd, Exeter, EX1 3PB, UK
[c] Central Veterinary Institute (CVI), Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands, The Netherlands

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ABSTRACT

Foot-and-mouth disease virus (FMDV) control measures rely on understanding of virus transmission mechanisms. Direct contact between naïve and infected animals or spread by contaminated fomites is prevented by quarantines and rigorous decontamination procedures during outbreaks. Transmission of FMDV by aerosol may not be prevented by these control measures and this route of transmission may allow infection of animals at distance from the infection source. Understanding the potential for aerosol spread of specific FMDV strains is important for informing control strategies in an outbreak. Here, the potential for transmission of an FMDV Asia 1 strain between pigs and cattle by indirect aerosol exposure was evaluated in an experimental setting. Four naïve calves were exposed to aerosols emitted from three infected pigs in an adjacent room for a 10 h period. Direct contact between pigs and cattle and fomite transfer between rooms was prevented. Viral titres in aerosols emitted by the infected pigs were measured to estimate the dose that calves were exposed to. One of the calves developed clinical signs of FMD, whilst there was serological evidence for spread to cattle by aerosol transmission in the remaining three calves. This highlights the possibility that this FMDV Asia 1 strain could be spread by aerosol transmission given appropriate environmental conditions should an outbreak occur in pigs. Our estimates suggest the exposure dose required for aerosol transmission was higher than has been previously quantified for other serotypes, implying that aerosols are less likely to play a significant role in transmission and spread of this FMDV strain.

1. Introduction

Foot-and-mouth disease virus (FMDV) affects cloven hoofed animals and the causative virus can spread rapidly. FMDV is an economically important disease of livestock, with infection resulting in loss of productivity and incurring costly control measures, such as restrictions on the movement and trade of animals and their products, and vaccination (Bergevoet and van Asseldonk, 2014). Understanding the modes of transmission of the virus will improve the effectiveness of control strategies for FMDV. Restrictions on the movement of livestock and decontamination procedures for farm sites (DEFRA, 2011) address spread of disease by direct contact and fomite transmission but do not mitigate the risk of spread of infection by airborne routes. A source producing large quantities of virus, combined with specific epidemiological and weather conditions are required for aerosol spread of FMDV to be viable (Hugh-Jones and Wright, 1970). Low turbulence air flow increases the likelihood of aerosol spread as it minimises the dilution of the aerosolised virus in the atmosphere, whilst high relative humidity (>55%) is required for the survival of the FMDV in air (Donaldson, 1972; Gloster et al., 1981).

Attempts to understand and predict the aerosol transmission of FMDV by statistical modelling incorporate data on viral excretions, virus plume characteristics, topography and meteorology to determine the extent of spread of virus in specific situations (Gloster et al., 1981; Schley et al., 2009). Despite these attempts, the contribution of aerosolised virus to short-distance spread of FMDV between animals remains poorly quantified. Increased understanding of this transmission mechanism may be valuable in developing epidemiological models for risk prediction as well as challenge models to study virus pathogenesis or to measure the efficacy of vaccines.

The potential for aerosol transmission of FMDV poses a significant risk in temperate climates and has previously been

* Corresponding author.
E-mail address: claire.colenutt@pirbright.ac.uk (C. Colenutt).

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implicated in the distribution pattern and spread of outbreaks in Europe (Hugh-Jones and Wright, 1970; Sellers and Forman, 1973; Donaldson et al., 1982a; Gloster et al., 1982). The capacity for aerosol transmission may be virus serotype or even strain specific. For example, experimental studies using isolates of the O PanAsia FMDV strain that caused the 2001 epidemics in the UK and the Netherlands (Aggarwal et al., 2002; Bouma et al., 2004) have suggested that the potential for aerosol transmission of this strain between ruminants was possible, but limited. In separate experiments, the potential of the O1 BFS 1860/UK/67 strain for aerosol transmission between cattle was demonstrated, where a 24 h challenge period resulted in aerosol transmission of the FMDV strain (Juleff et al., 2013).

The FMDV serotype Asia 1 has shown recent potential for epidemic spread (Valarcher et al., 2009) and has been reported in outbreaks in Turkey (Gilbert et al., 2005; Knight-Jones et al., 2014; FAO, 2014), a country which bridges the gap between endemic Middle East Asia and the FMD free countries of the Western Europe. FMDV serotypes circulating in this area could therefore be the source of an FMDV incursion into Europe. Knowledge of the transmission properties of the Asia 1 serotype would be valuable to assess the risk associated with any such incursion. Transmission of Asia 1 strains by direct contact has been studied experimentally (Pacheco et al., 2012; Bravo de Rueda et al., 2014), but information regarding the potential for aerosol spread of this serotype is limited.

Several methodologies have previously been used to directly deliver infectious FMDV aerosols (referred to as aerosol inoculation), including the use of cabinets containing infectious animals and nebulisers to aerosolise virus suspensions (Donaldson et al., 1987; Pacheco et al., 2010). These studies confirmed that inhaled virus aerosols can cause infection in cattle and also contributed to estimates of the doses required for infection and disease (Gonzales et al., 2014). However, such studies may not fully describe the aerosol transmission potential of a specific virus serotype under field conditions. For example, FMDV O UKG 2001 (O PanAsia strain) has been successfully used for aerosol inoculation using a nebulizer; in an experimental setting, calves became infected and sick when challenged with a total dose of approximately 10^3 $TCID_{50}$ (tissue culture infectious dose, titred in bovine thyroid (BTY) cells) in artificially produced aerosols (Parida, personal comm.). A total of 4 challenges were carried out and in all cases, aerosol inoculation with 10^3 $TCID_{50}$ was successful in causing disease in cattle. In contrast to this, when naïve calves were placed in adjacent pens to calves infected with the closely related O 2001 Dutch isolate, no transmission was observed (Bouma et al., 2004).

The objective of this study was to evaluate the potential aerosol transmission of an FMDV Asia 1 strain from pigs to cattle. To achieve this goal, infected pigs were used as a source of virus aerosols for the challenge of cattle. Procedures and physical separation of the animals, equipment and personnel ensured that the challenge was solely by aerosolised virus. Air flow was controlled so that emissions from the infected pigs were carried through the adjacent room housing the naïve cattle. This challenge method differs from aerosol inoculation in that the virus is not delivered directly to the naïve animals, therefore providing a more natural simulation of airborne infection in the field.

2. Methods

2.1. Animal experiments

Two experiments were performed; a pilot and a transmission experiment (see Sections 2.4.1 and 2.4.2). The pilot experiment was carried out with the aim to evaluate: i) the levels of virus likely to be generated by infected (source) pigs, ii) the experimental design for the transmission experiment, iii) air flow and virus (aerosol) carriage through these rooms and iv) the sampling approaches to be used to quantify FMD virus titre in aerosols.

Animal experimentation was approved by The Pirbright Institute (TPI) ethical review board under the authority of a Home Office project licence (PPL 70/7158) in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.2. Virus and challenge

A cattle strain of FMDV Asia 1 virus (HKN/05/2005) isolated from Hong Kong in 2005 was passaged once in bovine thyroid (BTY) cell culture (Snowdon, 1966). Thereafter, the virus was passaged once in cattle to derive the inoculum for the pilot and transmission study. Three cross-bred Landrace pigs (30 kg) were used as virus sources for each of the pilot and transmission experiments. Each pig was inoculated intradermally using three sites in the heel bulb of the right rear foot, with $10^{5.6}$ $TCID_{50}$ in a total of 0.2 mL (measured in BTY cells) on Day 0. No cattle were used in the pilot study. In the transmission experiment, four conventional naïve Holstein Friesian calves (six months old, ~150 kg) were used as recipients and challenged with virus aerosols generated by the three source pigs.

2.3. Experimental set up

Three consecutive, adjoining animal rooms within a high biosecurity, negative pressure building were utilised in the study. The first room, room A (Fig. 1), held the donor pigs for both the pilot and transmission study. When present, the cattle were kept in room B. Airtight doorways between the rooms allowed them to remain separate or be connected as required. Air flow to each room was controlled via separate ceiling-mounted inlet and extract ducts. Sampling positions are shown in Fig. 1. At position 1, samples were collected close to pigs, with instruments held between 10 cm and 60 cm away from the noses of individual animals. Other sampling positions were at increasing distances, but located so as to be in the path of the airstream from the pigs. Position 2 (3 m from infected pigs) was located in the doorway between room A and room B (Fig. 1). Positions 3 and 4 in rooms B and C were 5 m and 8 m away from the infected pigs, respectively.

To facilitate indirect aerosol exposure of cattle in the transmission experiment, doors between the rooms were removed

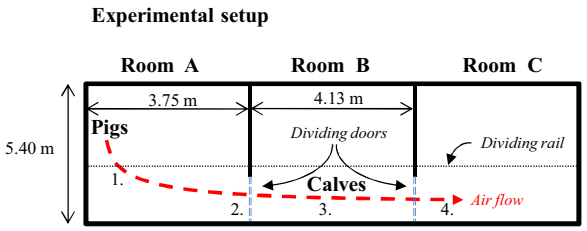

Fig. 1. Experimental set up for pilot study and transmission study. Numbers (1.–4.) represent position of air samplers. Position 1 represents samples taken in close proximity to infected pigs (10–60 cm from animals). Positions 2, 3 and 4 were 0.3–1.0 m above the ground, at approximately 3 m, 5 m and 8 m distance from the pigs respectively. Red dashed arrows demonstrate direction of air flow and blue dashed lines show doorways between animal rooms. A set of metal dividing rails kept animals in a particular part of the rooms with minimal effect on airflow. A set of mobile rails that formed a temporary pen to hold the pigs in position is not shown. Calves were only present in the transmission study. Dividing doors were opened for a fixed period of time in both the pilot and transmission study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)