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Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs

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SUMMARY

Foot-and-mouth disease virus (FMDV) can be spread by a variety of mechanisms, including wind. Simulation models, developed to predict the risk of airborne spread, have played an important part in decision making in some outbreaks. The amount of airborne virus excreted as well as the minimal infectious dose (MID) of FMDV for different species are important determinants of airborne spread. The objective of this study was to obtain data for the O_1 Lausanne, O SKR 2000 and O UKG 2001 strains of FMDV to enhance the capability of such models. Pigs were exposed to naturally generated aerosols of the three strains using an experimental design which delivered high doses of the two strains O_1 Lausanne and O SKR 2000 over a short period, or of the O UKG 2001 strain over an extended period. The average excretion of the O_1 Lausanne strain was $10^{6·4}$ $TCID_{50}$ per pig per hour. The excretion of the O SKR 2000 strain averaged $10^{5·8}$ and the O UKG 2001 strain $10^{6·1}$ TCID per pig per 24 h. The results show that the previous estimate of 'above' 800 $TCID_{50}$ as the MID50 for the O_1 Lausanne strain is a considerable under-estimate and that the real dose may be as high as 6000 $TCID_{50}$. A dose of around 650 $TCID_{50}$ of the O SKR 2000 strain failed to infect any pigs. Thus, the aerosol MID_{50} for pigs for this isolate is at least 1000 $TCID_{50}$ and likely to be as high or higher than the O_1 Lausanne strain. The exposure of pairs of recipient pigs kept physically separated from donor pigs in a series of rooms to aerosol exposure doses of the O UKG 2001 strain of around 50 $TCID_{50}$ per min for 24–48 h failed to infect any of eight pigs. Thus, the present experiment confirms our previous findings [1, 2] that pigs, compared to cattle and sheep, are relatively resistant to infection with airborne FMDV.

INTRODUCTION

FMDV is a viral disease of domesticated and wild ruminants and pigs characterized by the development of vesicles in and around the mouth and on the feet. FMDV is a member of the *Aphthovirus* genus within the *Picornaviridae* family [3]. FMDV is greatly feared by farmers and veterinary authorities because of its highly contagious nature and the difficulty of eradicating the virus. Countries or regions free of FMDV take severe measures to protect their status, such as maintaining embargoes against the importation of animals and products from countries considered a risk. Consequently, FMDV is the major disease constraint to international trade in livestock and animal products.

The contagious nature of FMDV is a reflection of a number of factors, including the wide host range of the virus, the high concentrations of virus excreted by infected animals, the low doses required to initiate infection and the multiplicity of routes by which the virus can initiate infection. FMDV is most often spread by the movement of infected animals. Next in frequency is spread by contaminated animal products, e.g. milk and meat. Infection may also be spread

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mechanically, for example by virus on vehicles, milking machines or on the hands of animal attendants. An additional mechanism is the spread of virus on the wind. This occurs infrequently as it requires particular climatic and epidemiological conditions. Windborne spread of the disease is uncontrollable and when it occurs can be dramatic [4, 5].

A critical determinant of the progression of an FMDV epidemic is the basic reproduction number, R_0 which can be defined as the number of secondary cases arising from the introduction of one primary case into a fully susceptible population. Values for R_0 can be obtained from the Parameters of *SLIR* models [6–8]. An analysis of 25 outbreaks in the United Kingdom between 1942 and 1967 gave a central value of $R_0 = 3{\cdot}5$. However, during the first 10 days of 1967–8 UK epidemic, when spread was by the wind [4, 5, 9–11], the R_0 values were 22–86. Airborne spread of FMDV, in addition to being rapid and extensive, can result in the transmission of infection beyond established disease control areas. For example, spread over a distance of 60 km over land and over 250 km over the sea have been recorded [4, 5]. Such long distance spread requires the emission of large amounts of airborne virus at source and a high density of susceptible animals downwind. Most often such spread occurs when large numbers of pigs are infected at source and cattle are present down-wind. Spread over long distances also requires particular climatic conditions and an FMD virus isolate which is excreted by infected animals in high amounts. An example is the C Noville strain which can be excreted at the rate of $10^{8{\cdot}6}$ $TCID_{50}$ per pig per 24 h [12]. However, recent experiments indicate that contemporary isolates of FMD virus serotype O, for instance O SKR 1/2000 and O UKG 34/2001, are excreted at much lower levels (reduced by 100 to 300-fold) and that the aerosol excretion by cattle and sheep is about 60-fold lower than in pigs [13].

Given the potential of FMDV for rapid spread it is essential that suspected cases are quickly reported and diagnosed and rapidly eliminated as otherwise there is very high risk of further spread. Traditionally, the method for eradicating FMDV is to slaughter the clinically affected and in-contact susceptible animals on the infected premises and to impose movement restrictions on the surrounding farms. These measures should stop the spread of FMDV by the movement of animals and products and eliminate carrier animals. However, there would still be the possibility of windborne spread of the virus. The determination of the biological parameters of the airborne spread of FMDV such as virus excretion, airborne virus survival, the quantitation of minimal infectious doses and the marrying of those factors with the physical determinants of airborne particle diffusion has provided the basis for the development of models which can predict the risk of airborne spread of FMDV [5, 11, 14–22]. A parameter which has not been quantified in sufficient detail, although the subject of recent preliminary findings [1, 2], is the minimal infectious dose 50% (MID_{50}) of airborne FMDV needed to infect pigs. In earlier work [23] estimates were made by exposing pigs to artificially generated aerosols of virus but these may not be valid as it is now recognized that the pathogenesis of FMD in animals exposed to artificially generated aerosols is markedly different from that in animals exposed to natural aerosols [19, 21]. Furthermore, the mouse assay system used by Terpstra [23] is less sensitive for quantifying FMD virus than the bovine thyroid monolayer cell culture system [24] and so the MID_{50} he calculated may have been underestimated. In our previous study [1], we examined the aerosol MID_{50} for pigs for the O_1 Lausanne virus, using a similar method to that used previously for determining the doses required to infect sheep and cattle [18, 19]. The studies indicated that a dose of more than 800 $TCID_{50}$ was required to cause clinical disease in 50% of exposed pigs. Although several pigs developed a transient antibody response, only a single pig out of 40 pigs exposed to virus developed clinical disease. Therefore, this estimate of more than 800 $TCID_{50}$ to cause disease is considered a conservative (minimum) estimate.

The objective of the present investigation was to increase the data for the MID50 of airborne virus using additional strains of FMDV delivered to pigs as natural aerosols as well as modified exposure arrangements making it possible to deliver high doses of virus to recipient pigs. We have extended the previous studies with the O_1 Lausanne strain and added two further strains of FMDV, the O SKR 2000 and the O UKG 2001 strains (both members of the type O PanAsia group of strains).

METHODS

Animals

The pigs were Landrace cross-bred Large White weighing between 20 and 30 kg at the start of the experiments. Three separate experiments (1–3) were

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done. Three 'donor' pigs, i.e. animals selected from a group of 4 inoculated animals as a source of natural aerosols of FMDV, and 8 or 10 'recipient' pigs, i.e. animals exposed to airborne FMDV, were used in each of Experiments 1 and 2. In Experiment 3, a total of 5 pigs were inoculated and then each transferred to a cubicle containing an uninoculated pig in a series of rooms. In the other cubicle in each of the rooms were 2 recipient pigs. Thus, there was direct contact between the inoculated and contact pigs, while the recipient pigs were exposed to aerosol virus generated within the room. Four pigs located in room 3 of Experiment 3 were excluded from the results because on two occasions a recipient pig managed to escape from its cubicle and climb into the cubicle with the donor pigs. Thus this animal was potentially exposed to direct transmission. Therefore, the results from Experiment 3 consist of the results from 4 donor pigs, 4 direct contacts and 8 recipient pigs.

All pigs were housed within cubicles in isolation rooms of a biosecure animal building as described previously [1]. Donor pigs were inoculated intradermally/subdermally in the heel bulbs of a left fore foot [25] with approximately 0·5 ml of stock virus No. 1 (O_1 Lausanne for Expt 1), stock virus No. 7 (O SKR 2000 passaged 3 times in pigs for Expt 2) or stock virus No. 9 (O UKG 34/2001 from a naturally infected field case of FMDV in a pig for Expt 3). All the inocula were diluted 1:10 in MEM-HEPES (Eagle's Minimal Essential Medium with 20 mM HEPES buffer and $\times 2$ antibiotics). Titration of the inocula showed that each animal received around $10^{5\cdot5}$ BTY $TCID_{50}$ of the O Lausanne inoculum, around $10^{5\cdot5}$ $TCID_{50}$ of the O SKR 2000 inoculum or around $10^{7\cdot5}$ $TCID_{50}$ of the O UKG 34/2001 isolate.

A clinical examination of the donor pigs for signs of FMD was carried out at least once and sometimes twice per day. Rectal temperatures were recorded daily. When early signs of generalized vesicular disease were present (2 or 3 days after inoculation) three pigs (Expts 1 and 2) were selected as donors, removed and placed in an aerosol production chamber located in the corridor outside the room. Donor pigs were killed soon after they had been removed from the aerosol production chamber (Expts 1 and 2) or for 24–48 h after showing the first vesicular lesions (Expt 3).

Recipient pigs were housed singly (Expts 1 and 2) or in pairs (Expt 3) in cubicles constructed within biosecure isolation rooms. Each of five rooms contained two cubicles as described previously [1]. The inside dimensions of a cubicle were: length 156 cm and width 174 cm. The wooden side and front of each cubicle was 73 cm in height. The front of each cubicle was raised to 120 cm by a removable wooden panel. There was a 30 cm gap between each pair of cubicles into which a 115 cm high heavy plastic mat was placed to prevent contact between the pigs in adjacent cubicles. A space of 2–3 cm was left at the front of each cubicle between the floor and the bottom of the panel for washing and cleaning purposes. The gaps between the side panels and the floor were sealed with silicone filler to prevent side-to-side seepage of fluid.

After each recipient pig had been exposed to airborne virus (Expts 1 and 2) it was returned to its cubicle and examined daily for signs of FMD over a 3-week period (see below). For Expt 3 the recipient pigs were not exposed in the chamber. Instead they were exposed to the virus emitted over a 24–48 h period by the inoculated and contact donor pigs in the other cubicle in the room. The pigs were not handled except on the occasions when blood or nasal samples were being collected. Any animal which developed clinical signs of FMD was killed immediately, otherwise they were killed at the end of the experiments, i.e. at 20 or 21 days post exposure (d.p.e.).

Samples of epithelial tissue were collected from any animal which developed lesions and tested by ELISA (26–29) to confirm the presence of FMDV antigen.

Virus

The O_1 Lausanne Sw/65 strain of FMDV was obtained from the International Vaccine Bank at IAH, Pirbright. It had been passed in cattle by intradermolingual inoculation and then grown in IB-RS-2 cells [30, 31]. The titre of this stock virus (No. 1) was $10^{6\cdot7}$ $TCID_{50}$ when assayed in primary bovine thyroid (BTY) cells and $10^{5\cdot7}$ $TCID_{50}$ in IB-RS-2 cells. This stock virus (No. 1) was used for Expt 1 and is the same inoculum as in Expts 1–5 reported previously [1].

The virus used for Expt 2 was prepared by passing an original epithelial suspension of isolate O SKR 1/2000 three times in pigs, the initial passage being by needle inoculation and the other two passages by direct contact. A 10% (w/v) suspension of foot vesicular epithelial tissue lesion from the third passage was made in MEM-HEPES and stored as 0·5 ml aliquots at -70 °C. The titres of this stock virus (No. 7) were $10^{6\cdot45}$ and $10^{5\cdot7}$ $TCID_{50}$ per ml in BTY and IB-RS-2 cells, respectively.

The virus used for Expt 3 was prepared as an original suspension of vesicular epithelium collected

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from a pig at Brentwood Abattoir, Essex during the 2001 epidemic in the United Kingdom. The virus isolate is denoted FMDV O UKG 34/2001. A 10% (w/v) suspension of foot vesicular epithelial tissue lesion was made in MEM-HEPES and stored as 0·5 ml aliquots at −70 °C. The titres of this stock virus (No. 9) were $10^{8·8}$ and $10^{7·6}$ $TCID_{60}$ per ml in BTY and IB-RS-2 cells, respectively.

Exposure of pigs to natural aerosols of FMD virus

The procedures used were modifications of those described previously for determining the minimum infectious doses of FMDV for both pigs, cattle and sheep [1, 18, 19]. In brief, three donor pigs which were the source of natural aerosol for Expts 1 and 2 were selected at 2–3 d.p.i. when they had signs of early generalized FMD and placed in the 610 litre aerosol production chamber [5]. The pigs were lethargic, lay down and remained recumbent on the floor of the chamber. The chamber was closed and the personnel who had been in contact with the pigs thoroughly cleansed and disinfected their hands, protective clothing, boots, the outside of the chamber and the surrounding area. The chamber was then moved to the other end of the corridor where two exposure masks connected to 30 cm long, 2·5 cm wide tubing were attached to its side.

Before exposure to airborne virus a pair of recipient pigs were taken individually, placed on their backs on a wooden cradle, blood-sampled from the anterior vena cava and then sedated by injection with Propofol (Rapinovet 10 mg/ml, Schering-Plough Animal Health, Welwyn Garden City, UK) into the anterior vena cava at a dose rate of 2 mg per kg body weight. The pair of sedated recipient pigs were then connected to the chamber via the exposure masks and allowed to inhale airborne virus for 5 min. During the exposure period the transmission tunnel used in previous experiments [1] was disconnected from the cabinet so the only fresh air drawn into the cabinet was that which entered through a small hole in one side of the chamber. The resulting challenge concentrations of airborne virus were much higher than in the previous experiments. After exposure to virus the recipient pigs were transferred to individual cubicles in biosecure isolation rooms [1]. Two experiments (1 and 2), using a series of 8 and 10 pigs in each, respectively, were performed. In the interval between the exposure of each pair of recipient pigs fresh air was drawn through the cabinet by connecting it to wide-bore ducting (15 cm internal diameter × 18 metres in length) secured just beneath the filter housing of an extractor air vent in the ceiling of the corridor.

The amount of air inspired during the exposure period was based on previous experiments, which showed that the average volume of air inspired by a pig under these experimental conditions (measured by an ultrasonic flowmeter) was around 0·6 litre air per kg per min. This estimate was based on the individual measurement of 39 pigs of 20–30 kg of weight [1].

The humidity in the cabinet and in the rooms (Expt 3), monitored by an electronic humidity meter (Airflow Developments Ltd, High Wycombe, Bucks HP12 3QP, UK), was raised above 60% relative humidity by spraying water onto the floor of the corridor or walls of the rooms.

The experimental design for Expt 3 was different. Recipient pigs, two per cubicle, in a series of four isolation rooms were exposed to airborne virus generated by a pair of inoculated/direct contact pigs in the other cubicles in the rooms. The inoculated/direct contact pigs were present in the rooms from when the donor pigs were inoculated until 24–48 h after they had developed clinical signs. Both donor pigs were then removed and killed. The amounts of virus in the air to which recipients were exposed were estimated by collecting air samples using a cyclone sampler as well as by placing donor (inoculated and contact) pigs in the cabinet described above and collecting multiple air samples with a 3-stage (May) sampler.

After exposure, each recipient pig was returned to its cubicle (Expts 1 and 2) or left in the cubicle (Expt 3) and observed daily for signs of FMD. In order to avoid mechanical transfer of virus the pigs were only handled when blood and swab samples were collected or when they developed signs of FMD. Any recipient pig which developed signs of FMD was removed from its cubicle and killed. Blood samples were collected from recipient pigs at 14 and 20 or 21 d.p.e. and nasal swabs for virus isolation at 7 days d.p.e. (Expts 1 and 2).

Air sampling methods

Air samples were collected from the corridor to test for the presence of background virus after the donor pigs had been placed in the aerosol production chamber and after the last recipient pig had been

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exposed to airborne virus (Expt 1 and 2). In Expt 3 air samples were also collected from two isolation rooms each containing an inoculated, a direct contact and two recipient pigs. Sampling was done with an all-glass cyclone sampler operating for 2 min (Expts 1 and 2) or 20 min (Expt 3) at a sampling rate of around 170 litres/min [18].

During the exposure of each pair of recipient pigs (Expts 1 and 2) an air sample was collected from the aerosol production cabinet using a 3-stage liquid impinger [32] with a total of 30 ml collecting fluid and operating at 55 litres/min for 5 min. In Expt 3 two samples were collected from the cabinet with the same sampler when three donor pigs taken from each of two isolation rooms were placed in it.

The collecting fluid used in both samplers was MEM-HEPES with antibiotics and BSA added to 0·1 % [18, 19]. The pigs were exposed to virus through ports in the back of the cabinet. Air samples were collected with the 3-stage sampler through a port in one end of the cabinet.

Measurement and recording of respiration

The respiratory function (volume) of each recipient pig was estimated by referring to previous findings [1] where detailed measurements of 39 pigs were made with an ultrasonic phase-shift respiratory flowmeter (BRDL Flowmetrics, Birmingham) [33, 34] The volume of respired air was calculated as the average of calculated inspiration and expiration in order to minimize any fluctuations caused by leaks or uneven flow. Values from 39 pigs of 20–30 kg of weight indicated that respiration equals about 0·6 litre air per kg per min [1]. The dose inhaled by each pig was determined by multiplying the calculated volume of respiration during exposure by the concentration of virus per litre of air as calculated from the air samples. The latter were obtained from the end-point titration of virus in the collecting fluid of the particular air-sampler used, multiplied by the volume of the collecting fluid and the flow rate of the sampler.

Assay for virus

The infectivity in the collection fluid from air samplers, in blood samples and nasal swabs were assayed by inoculating monolayer cultures of primary bovine thyroid (BTY) cells in roller tubes [18, 19, 24]. Tenfold dilution series of collecting fluid samples were made and each dilution was inoculated onto five tubes. For the assay of virus in blood and nasal swabs each sample in the dilution series was inoculated onto 3 BTY tubes. The specificity of the cytopathic effect observed in cell cultures was confirmed by antigen ELISA [26–29].

Assay for antibodies

Serum samples were tested for the presence of antibodies to FMDV by an enzyme-linked immunosorbent assay (ELISA) [35–39].

RESULTS

Airborne virus recovery and estimated respiration and exposure doses

The amount of virus in air samples, the concentration of virus in the air, the volumes of air estimated to be inhaled by the pigs and the total dose to which the pigs were exposed in each experiment are shown in Tables 1–3. The average dose a pig received in each experiment was calculated by: (i) adding the measurable amounts of virus received by the recipient pigs in the experiment; and (ii) dividing that sum by the number of those pigs.

In brief, the data shown in Tables 1–3 can be summarized as follows:

Expt 1

8 pigs receiving an average dose of 1700 $TCID_{50}$ during a 5 min exposure period (340 $TCID_{50}$ per min).

Expt 2

10 pigs receiving an average dose of 650 $TCID_{50}$ during a 5 min exposure period (130 $TCID_{50}$ per min).

Expt 3

8 pigs receiving an average dose of 50 $TCID_{50}$ per min for at least 24 h (accumulated dose of more than 70000 $TCID_{50}$).

The respired volume for each pig was calculated as the average of calculated inspiration and expiration in order to minimize any variation caused by leaks or uneven flow through the aerosol delivery system.

Based on the excretion of airborne virus from the donor pigs, we have calculated that the average excretion of FMDV O_1 Lausanne equals $10^{6·4}$ $TCID_{50}$ per 24 h period per adult pig (calculated as a pig of around 90–100 kg, which equates to three small donor

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Table 1. *Doses of airborne O_1 Lausanne virus inhaled by recipient pigs in Expt 1* (*all exposed 5 min*)

Pig no.	Calc inspiration Mean*/5 min litres	Air sample $TCID_{50}$/30 ml 2×55 litres air	$TCID_{50}$/litre air	Total dose inhaled $TCID_{50}$
UG77	72	May 1: $10^2 \times 30$	27	1944
UG78	73			1971
UG79	63	May 2; $10^{2\cdot4} \times 30$	70	4410
UG80	48			3360
UG81	60	May 3; $10^{1\cdot8} \times 30$	17	1020
UG82	70			1190
UG83	68	May 4; $10^{0\cdot4} \times 30$	0·7	50
UG84	54			40

Four air-samples were collected with May samplers for 2 min at a sampling rate of 55 l/min. Pre- and post-exposure samples of the background air in the corridor (cyclone sampler operating from 2 min at 170 l/ml. Average volume of inspired (l/min per kg was $0{\cdot}6 \pm 0{\cdot}2$ (sd) calculated from [1].
Average dose per pig in this experiment was around 1700 $TCID_{50}$.

Table 2. *Doses of airborne O SKR 1/2000 virus inhaled by recipient pigs in Expt 2* (*all exposed 5 min*)

Pig no.	Calc inspiration Mean*/5 min litres	Air sample $TCID_{50}$/10 ml 5×55 litre air	$TCID_{50}$/litre air	Total dose inhaled $TCID_{50}$
UI27	80	May 1: 1·5	3·45	275 TCID
UI26	80			275 TCID
UI25	80	May 2: 2·1	13·04	1040 TCID
UI24	80			1150 TCID
UI23	80	May 3: 2·1	13·04	1040 TCID
UI22	80			1040 TCID
UI21	80	May 4: 1·6	4·11	330 TCID
UI20	80			330 TCID
UI19	80	May 5: 1·8	7·38	590 TCID
UI18	80			590 TCID

Average volume of inspired calculated from [1].
Average dose per pig in this experiment was around 650 $TCID_{50}$.

pigs). The excretion of O SKR 2000 averaged $10^{5\cdot8}$ and the O UKG strain $10^{6\cdot1}$ $TCID_{50}$ per 24 h per pig (90–100 kg). In Expt 3 the amount of virus to which recipient pigs were exposed equated to $10^{5\cdot5}$ $TCID_{50}$ per 24 h per room. This lower challenge dose was due to the continued operation of the ventilation in the rooms which was around three air-changes per hour.

Air sampling of corridor air

Samples of air from the corridor collected before and after the exposure of the recipient pigs were negative for virus in Expt 1 but positive in Expt 2. However, the quantities of virus were low; 2·5 $TCID_{50}$/litre in the sample before exposure and 0·08 $TCID_{50}$/litre in the sample after exposure, compared to the concentration of around 8 $TCID_{50}$/litre in the cabinet inhaled by the recipient pigs during exposure.

Clinical signs, viraemia and seroconversion

The only recipient pig which developed FMDV was No. UG 77 in Expt 1. At 4 d.p.e. it had painful feet and vesicles on the snout and on the coronary bands

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Table 3. *Doses of airborne O UKG 34/2001 virus excreted by donors and inhaled by recipient pigs in Expt 3*

Pig no.	Air sample ($TCID_{50}$/ml in 30 ml 5×55 (275) litre air)	$TCID_{50}$/litre air	Excreted in 5 min ($TCID_{50}$)	Excreted 24 h ($Log_{10}/TCID_{50}$)
(A) Excretion				
UJ16 UJ17 UJ23	May 1: 2·2	17·29	4750	6·14
UJ16 UJ17 UJ23	May 2: 1·8	6·87	1889	5·7
UJ17 UJ23 UJ26	May 3: 2·6	43·43	11940	6·54
UJ21 UJ23 UJ26	May 4: 1·4	2·73	751	5·3
Average May sampler estimate of virus excretion is $Log_{10}^{6·14}$ $TCID_{50}$ per 24 h per 3 small pigs (equivalent to a single pig around 90–100 kg)				
(B) Inhaled virus				
UJ16 UJ23 UJ21 UJ22	Cyclone 1: 2·6	2·49	3980 $TCID_{50}$	5·46 logs $TCID_{50}$
UJ17 UJ26 UJ24 UJ25	Cyclone 2: 2·6	2·49	3980 $TCID_{50}$	5·46 logs $TCID_{50}$
	Average Cyclone is 5·46 logs per 24 h			

Four air samples were collected with May samplers for 5 min at a sampling rate of 55 l/min. Cyclone sampling was directly in the box with infected pigs and the sampler operating for 20 min at 170 l/min). Average volume of inspired (l/min per kg was $0·6 \pm 0·2$ (sd) calculated from [1].

Average dose per pig in this experiment was around 45–50 $TCID_{50}$ per minute (breathing 18–20 l air per minute per pig). Note that the concentrations of virus in the boxes (B. Measured by the Cyclone samplers) were reduced in comparison to the concentration in the exposure cabinet, A. measured by the May samplers. The difference correlates to approximately 0·7 logs or a reduction of the concentration in the room of around five fold. This was expected as the ventilation system of the rooms had been reduced to 3–5 air changes per hour.

of the feet. It was killed immediately. Post-mortem examination showed that it had vesicular lesions on all four feet, the gingival mucosa, the tongue and snout. No other gross pathological lesions were found.

Histopathological examination of haematoxylin and eosin stained sections confirmed the presence of vesicular lesions but no other microscopic lesions. Samples of blood and epithelial tissue collected at 4 d.p.e. contained high titres of FMDV.

None of the other seven recipient pigs in Expt 1 nor any of those in Expts 2 or 3 developed signs of disease. Nasal swabs taken at 7 and 21 d.p.e. (Expts 1 and 2) were negative for FMDV by cell culture and by RT-PCR. Blood samples taken at 7, 10, 14 and 21 d.p.e. (Expt 1) showed antibodies to FMDV in 4 out of the remaining 7 recipients (Table 4), specifically at 10 or 14 d.p.e. (Table 4). Thus, of 8 pigs exposed to a very high dose of virus (Table 1), 1 developed typical signs of FMDV and 4 were subclinically infected (Table 4). Interestingly, by 21 d.p.e. those pigs were negative for serum antibody, indicating as seen previously [1, 40] that they had experienced an infection of very short duration.

Antibodies were not detected in any of the recipient pigs in Expts 2 and 3 (data not shown), except for a single pig (UJ 28) in Expt 3 which had a borderline ELISA titre of 22 at 14 d.p.e. and 45 on day 20 suggesting that this animal might have had a transient infection. However, the pigs in this room (No. 3) were excluded from the experiment because there had been

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Table 4. *Assay of sera from recipient pigs in Expt 1 by liquid-phase-blocking-ELISA*

Pig	Prebleed and 7 d.p.e.	Antibody titre* Blood 10 d.p.e.	14 d.p.e.	21 d.p.e.
UG77	neg (< 16)	neg†		
UG78	neg	neg	90	neg
UG79	neg	neg	64	neg
UG80	neg	neg	neg	neg
UG81	neg	90	90	neg
UG82	neg	32	32	neg
UG83	neg	neg	64	neg
UG84	neg	neg	22	neg

* A titre of > 40 is considered positive [43].
† When killed 4 d.p.e., N.B. clinical disease.

brief direct contact between this recipient pig and the inoculated donor pig and all four pigs in this box were excluded from the experiment.

The results can be summarized as follows:

Expt 1: 8 pigs receiving an average dose of 1700 $TCID_{50}$ during a 5 min exposure period (340 $TCID_{50}$ per min). One pig developed clinical FMD, 4 pigs were subclinically infected and 3 remained normal. Thus, the MID_{50} dose to subclinically infect the pigs in this experiment with the O_1 Lausanne strain of virus is even higher than the dose reported in an earlier study (1) and may be around 1500 $TCID_{50}$ (calculated after Kärber (as described in [41]). The dose to cause clinical disease may be as high as 4000–6000 $TCID_{50}$ when given during a 5 min period.

Expt 2: 10 pigs received an average dose of 650 $TCID_{50}$ during a 5 min exposure period (130 $TCID_{50}$ per min). None of the pigs developed FMDV or detectable antibodies. Since none of the pigs developed infection or disease, it is difficult to calculate an accurate MID for the O SKR 2000 strain. However, from the limited data, it appears that the MID_{50} dose to cause either subclinical infection or disease is likely to be more than 1000 $TCID_{50}$ for this strain and is likely to be as high or higher than the O_1 Lausanne isolate.

Expt 3: 8 pigs receiving an average dose of 50 $TCID_{50}$ per min for at least 24 h which equates to an accumulated dose of more than 70000 $TCID_{50}$. None of the pigs developed FMD or detectable antibodies. Thus, when accumulated over a 24 h period, it seems that the MID_{50} dose to infect pigs with the UKG isolate may be higher than 70000 $TCID_{50}$. Thus, a concentration of around 2500 $TCID_{50}$ per m^3 (as found in the experiment) is apparently not sufficient to infect pigs with this strain, even when they were exposed for 24 h or more.

DISCUSSION

The primary objective of this study was to define more accurately the minimal infectious dose (MID) for pigs of FMDV inhaled as a natural aerosol. Although we have previously determined the dose for the O_1 Lausanne strain [1] only one pig developed clinical disease so more studies were needed with higher challenge doses and with different strains of virus. The secondary objective was to generate data to enhance the capability of the computer-based model Rimpuff [22], an atmospheric model that can predict the risk of airborne FMDV. Rimpuff can predict the risk for cattle and sheep downwind of a source of virus emission but not with accuracy for pigs since only limited data are available for the MID_{50} for that species.

The results show that of the 26 pigs exposed to airborne virus in Expts 1–3, 4 were subclinically infected and only 1 developed typical signs of FMD. The infected pigs were all in the group exposed to the O_1 Lausanne strain (Expt 1), the virus excreted in the greatest quantities by donor pigs and therefore presenting the most severe challenge to recipient pigs which were calculated to have inspired around 340 $TCID_{50}$ per min for 5 min. This dose is 5–10 times greater than in earlier experiments with the same strain when pigs inspired around 30 $TCID_{50}$ per minute for 10 min [1]. This may indicate, that the previous experimental design which involved the delivery of airborne virus along a wind tunnel may have favoured the delivery of a higher proportion of larger particles than in the present study where the larger particles would have had greater opportunity to sediment. Alternatively, the previously estimated MID_{50} value of above 800 $TCID_{50}$ may have been a considerable underestimation and the real value could be much higher, perhaps as high as 6000 $TCID_{50}$. Pigs exposed to the O SKR 2000 strain and calculated to have inspired about 130 $TCID_{50}$ per min for 5 min did not develop clinical disease or subclinical infection. However, because donor pigs infected with the O SKR 2000 strain excreted relatively little virus ($10^{5.8}$ log $TCID_{50}$ per 24 h per pig compared to $10^{6.4}$ $TCID_{50}$ for the O_1 Lausanne virus) we were unable to increase the exposure concentration for the O SKR 2000 strain. In Expt 3 donor pigs infected with the UKG 34/2001 strain excreted around $10^{6.1}$ $TCID_{50}$ per pig

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per 24 h. In Expt 3 none of the recipient pigs in cubicles exposed to this strain at a concentration of around 50 $TCID_{50}$ per min i.e. an accumulated dose of approximately 70000 $TCID_{50}$ per pig in a > 24 h exposure period was infected. This indicates that the respiratory clearance mechanisms for FMDV inhaled by pigs are very efficient and that aerosols of FMDV have to be at very high concentrations to infect pigs. Also, a single recipient had transient contact to a donor pig and were excluded from the study together with the three other pigs in that particular box. However, this recipient pig shows that the antibody based assay works fine, in this case to indicate that contact transmission can be detected relatively easily even after very brief contact and most likely mediated by an aerosol consisting of rather large particles with a relatively high concentration of virus.

The findings in the present paper is supported by our previous study on the O_1 Lausanne strain [1]; experimental findings using the A_5 Parma strain of FMDV (G. O. Denney, unpublished results); and by another experiment with O UKG 2001 strain performed at IAH, Pirbright (N. Aggarwal, R. P. Kitching, unpublished results). Also relevant are field observations by veterinarians in the Philippines who have reported that FMDV rarely spreads from one pig premises to another when the possibility of direct contact can be excluded (C. Beningo, personal communication).

We conclude from the present and previous findings [1] that pigs, compared to sheep and cattle, are relatively resistant to infection by airborne FMDV. The doses required to cause infection and disease in pigs may be as high as 300–2000 and 800–6000 $TCID_{50}$, respectively. Furthermore, these doses need to be delivered within a very short period. By contrast, cattle and sheep can be infected by a dose of only 10 $TCID_{50}$ [18, 19]. Therefore, although a pig excrete as much virus as 60 sheep or cattle [13], it is very unlikely that an infected pig premises will generate a virus plume of sufficient concentration to cause aerosol infection of pigs located on separate farms. In fact our calculations indicate, that even though the excretion from pigs is about 60-fold higher than from sheep and cattle (for the UKG 2001 isolate [13] pigs are also at least 60 times (and probably more) as resistant to aerosol infection as sheep and cattle. Thus, the risk of airborne transmission from infected pigs to other pigs is probably low and only likely to occur at very short distances, similar to what we expect for ruminant to ruminant transmission by aerosol [13]. However, the combination of high excretion of aerosol virus from pigs with the high sensitivity of cattle and sheep by this route, makes this the main mode of airborne transmission of FMDV.

The exposure of pigs, cattle and sheep to low doses of airborne FMDV often results in sub-clinical infection. The transient antibody responses found in sub-clinically infected pigs in Expt 1 and the absence of detectable virus at 7 d.p.e. suggests that virus circulation ceased before infection progressed to cause clinical disease. It is probable that this early antibody response was primarily due to IgM [40].

It is theoretically possible that the exposure of pigs to a fraction of a MID_{50} could result in a proportion of the animals becoming infected [41, 42]. Those animals could then amplify the virus and transmit it to others either directly or indirectly. However, none of the 10 pigs exposed to 650 $TCID_{50}$ of the O SKR 2000 strain (Expt 2) or the 8 pigs exposed to 50 $TCID_{50}$ per min for 24 h (O UKG 2001 strain, Expt 3) became infected, which suggests that there is a threshold level below which infection does not occur, or more likely, where the respiratory clearance of the pig can prevent the establishment of FMDV infection.

It is not possible to compare the results in the study directly with those reported previously by Terpstra [23] since he used artificially generated aerosols which are known to cause an atypical infection [19, 21]. Also the mouse assay system used by Terpstra is much less sensitive than the BTY cell system for detecting FMDV [15]. Furthermore, the number of animals infected in Terpstra's experiments could have been under-estimated since all the animals he challenged were killed soon after exposure.

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