

# Natural aerosol transmission of foot-and-mouth disease virus to pigs: minimal infectious dose for strain O<sub>1</sub> Lausanne

S. ALEXANDERSEN\*, I. BROTHERHOOD AND A. I. DONALDSON

*Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK*

(Accepted 4 June 2001)

## SUMMARY

Foot-and-mouth disease virus (FMDV) can spread by a variety of mechanisms, including, under certain circumstances, by the wind. Simulation models have been developed to predict the risk of airborne spread of FMDV and have played an important part in decision making during emergencies. The minimal infectious dose of FMDV for different species by inhalation is an important determinant of airborne spread. Whereas the doses for cattle and sheep have been quantified, those for pigs are not known. The objective of the study was to obtain that data in order to enhance the capability of simulation models. Under experimental conditions, forty pigs were exposed individually to naturally generated aerosols of FMDV, strain O<sub>1</sub> Lausanne. The results indicated that doses under 100 TCID<sub>50</sub> failed to infect pigs but doses of approximately 300 TCID<sub>50</sub> caused short-term sub-clinical infection. The calculations suggested that a dose of more than 800 TCID<sub>50</sub> is required to cause infection and typical disease.

## INTRODUCTION

Foot-and-mouth disease virus (FMDV) is a viral disease of domesticated and wild cloven-hoofed animals characterized by the development of vesicles in and around the mouth and on the feet. It is caused by a member of the *Aphthovirus* genus within the *Picornaviridae* family [1]. FMDV is much feared by farmers and veterinary authorities because of its highly contagious nature and the difficulty to eradicate the virus. Countries free of FMDV go to great lengths to protect their status, such as placing embargoes against imports of animals and products from countries considered to pose a risk. As a consequence of those measures 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 its wide host range, the high concentrations of virus which infected animals excrete, the low doses required to initiate infection and the multiplicity of routes by which the virus can

initiate infection. FMDV is most commonly spread by the movement of infected animals. Next in frequency is spread by contaminated animal products, e.g. milk and meat. Infection may be spread by mechanical means also, for example, when animals contact virus on the surfaces of transport vehicles, milking machines or on the hands of animal attendants. An additional mechanism is the spread of FMDV by the wind. This occurs infrequently as it requires particular climatic and epidemiological conditions. However, spread by this means, when it occurs, can be dramatic [2, 3]

A critical determinant of the progression of FMDV epidemics 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 [4–6]. An analysis of 25 outbreaks in the United Kingdom between 1942 and 1967 gave a central value of  $R_0 = 3.5$ . However, the same analysis applied to the first 10 days of the 1967–8 UK epidemic, when spread was attributed to the transmission of virus by the wind [2, 3, 7–9], gave  $R_0$  values which ranged from 22 to 86.

\* Author for correspondence.

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 [2, 3].

Given the potential of FMDV for rapid spread it is essential that suspected cases are quickly reported, diagnosed and eliminated as otherwise there is a very high risk that epidemics will result. 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 virus by the movement of animals and products and eliminate carrier animals but leave open the possibility of wind-borne spread of the virus. The determination and expression of the biological determinants of the airborne spread of FMDV such as virus excretion, airborne virus survival and minimal infectious doses in quantitative terms and the marrying of those with the physical determinants of airborne particle diffusion have resulted in the development of mathematical models which can predict the risk of airborne spread of FMDV [3, 9–18]. A parameter which has not been quantified, however, is the minimal infectious dose 50% (MID<sub>50</sub>) of airborne FMDV for pigs. Estimates have been made by exposing pigs to artificially generated aerosols of virus [19] but these may not be valid as it is now recognized that the pathogenesis of FMDV in animals exposed to artificially generated aerosols is markedly different from that in animals exposed to natural aerosols [15, 17]. Furthermore, the mouse assay system used by Terpstra [19] is less sensitive for quantifying FMDV than is the bovine thyroid monolayer cell culture system [20] and so the MID<sub>50</sub> he calculated may have been underestimated.

The objective of the present investigation was to establish the MID<sub>50</sub> of airborne FMDV delivered to experimental pigs as a natural aerosol so that the values obtained can be used as input data for the atmospheric prediction model Rimpuff to improve and refine its capability [16, 18].

## METHODS

### Virus

The O<sub>1</sub> Lausanne Sw/65 strain of FMDV was obtained from the International Vaccine Bank at the

Institute for Animal Health, Pirbright. It had been passed in cattle by intra-dermolingual inoculation and then grown in IB-RS-2 cells [21]. It was received as 1 ml aliquots of ether-treated, cell culture maintenance fluid, containing 50% glycerol. The titre of this stock virus (No. 1) was 10<sup>6.7</sup> TCID<sub>50</sub> when assayed in primary bovine thyroid (BTY) cells and 10<sup>5.7</sup> in IB-RS-2 cells. This stock virus (No. 1) was used for Expts 1 and 5.

The virus used for Expts 2–4 was prepared by passing stock virus No. 1 three times in pigs. A 10% (w/v) suspension of foot vesicular epithelial tissue lesion from the third passage was made in MEM–HEPES and stored as 1 ml aliquots at –70 °C. The titre of this stock virus (No. 2) was 10<sup>8.7</sup> and 10<sup>7.2</sup> TCID<sub>50</sub> per ml in BTY and IB-RS-2 cells, respectively.

### Animals

Five separate experiments were performed. Two or 3 ‘donor’ pigs, i.e. animals selected from a group of 4 inoculated animals as a source of natural aerosols of FMDV, and 5 or 10 ‘recipient’ pigs, i.e. animals exposed to airborne FMDV, were used in each experiment. The pigs were predominantly Landrace cross-bred Large White but a few were partly of the Duroc or Hampshire breeds. They weighed between 15 and 31 kg at the start of the experiments.

The donor pigs were housed as a group within an isolation room of a biosecure animal building. In Expts 1 and 5 each of the donor pigs was inoculated intradermally in the heel bulbs of a left fore foot [22] with approx. 0.2 ml of stock virus No. 1 diluted 1:10 in MEM–HEPES (Eagle’s Minimal Essential Medium with 20 mM HEPES buffer and ×2 antibiotics). Titration of the inoculum showed that each animal received around 10<sup>5</sup> BTY TCID<sub>50</sub>. The pigs in the donor groups for Expts 2–4 were infected with stock virus No. 2 diluted 1:10 in MEM–HEPES but the volume of the inoculum was increased to around 0.5 ml and it was given by a combination of intradermal and subdermal injection in the heel bulbs of the left fore foot. The dose per pig was around 10<sup>7.0</sup> BTY TCID<sub>50</sub>.

A clinical examination of the donor pigs for signs of FMDV 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 either two (Expt 1) or three (Expts 2–5) pigs were selected as donors, removed from the room

and placed in an aerosol production chamber located immediately outside in the corridor. For Expts 1 and 5 the donor pigs were selected at 3 days post inoculation (d.p.i.), in Expts 2 and 3 they were selected at 2 d.p.i. and for Expt 4 a combination was used for two donor pigs at 3 d.p.i. and one donor pig at 2 d.p.i. Donor pigs were killed soon after they had been removed from the aerosol production chamber.

Recipient pigs were housed singly in cubicles constructed within biosecure isolation rooms. Each of the five rooms contained two cubicles. The walls of the room formed the back and one side of a cubicle. The opposite side and front of a cubicle were constructed from 25 mm thick marine plywood panels. The inside dimensions of a cubicle were: length 156 cm; width 174 cm. The wooden side and front of each cubicle were 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 was exposed to airborne virus it was returned to its cubicle and examined daily for signs of FMD over a 3-week period (see below). The pigs were not handled other than 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 at 21 or 22 d.p.e.

Samples of epithelial tissue were collected from any animal which developed lesions and tested by ELISA [23, 24] to confirm the presence of FMDV antigen.

### Exposure of pigs to natural aerosols of FMDV

The procedures used were similar to those described previously for determining the minimum infectious doses of FMDV for cattle and sheep using donor pigs to produce natural aerosols containing FMDV [14, 15]. However, some aspects were changed to adapt the method for use with pigs as recipients and to take advantage of recent technological advances.

At 3 d.p.i. (Expts 1, 5 and partly 4) or 2 d.p.i. (Expt 2, 3 and partly 4) when clinical signs of early generalized vesicular FMD were evident in the pigs, either two (Expt 1) or three (Expts 2–5) animals were

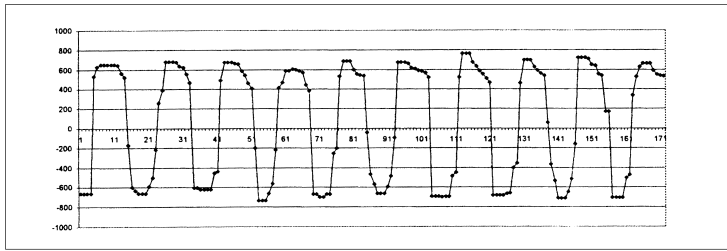
selected and placed in a 610 litre aerosol production chamber located in the corridor immediately outside the isolation room. The pigs were lethargic, lay down and remained recumbent on the floor of the chamber. The chamber was closed and the personnel who had been in contact with the pigs thoroughly 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 and connected to an 'exposure tunnel' comprised of wide-bore ducting (15 cm internal diameter  $\times$  18 m in length) which terminated in a metal housing containing an iris diaphragm flow valve. The end of the tunnel was secured just beneath the filter housing of an extractor air vent in the ceiling of the corridor.

The pressure in the corridor was negative relative to the rest of the building and therefore the air was drawn in a steady one-way direction into the aerosol production chamber, along the tunnel and then out through the extractor vent. The rate of flow of air through the aerosol delivery system was controlled by a screw valve at the entry port to the isolator chamber, a flap valve in the central part of the tunnel and an iris diaphragm at the end. The air flow through the tunnel was adjusted to between 0.25 and 0.5 m/s by moving the iris diaphragm and flap valve and taking readings with an electronic anemometer (Airflow Model TA-2, Surrey, UK).

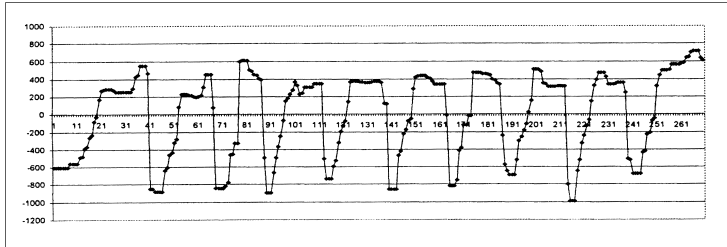
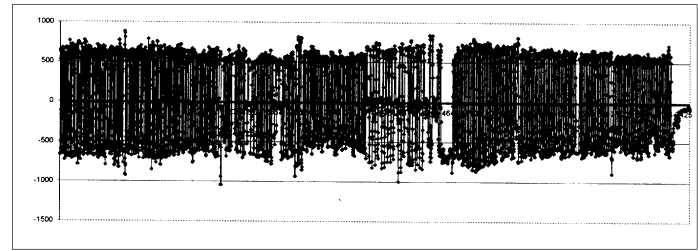
Before exposure to airborne virus each recipient pig was taken individually, placed on its back on a wooden cradle and blood-sampled from the anterior vena cava. The animal was then sedated by injection with Propofol (Rapinovel 10 mg/ml, Schering-Plough Animal Health, Welwyn Garden City, UK) into the anterior vena cava at a dose rate of 2 mg per kg body weight and fitted with a rubber mask which was connected to the exposure tunnel via an ultrasonic flowmeter.

The humidity in the tunnel was raised above ambient in Expts 3–5 by introducing a fine mist of water vapor generated by a DeVilbiss model 65 (Sommerset, PA, USA) ultrasonic nebulizer into the aerosol production chamber. The relative humidity was monitored by inserting the probe of an electronic humidity meter (Airflow Developments Ltd, High Wycombe, Bucks HP12 3QP, UK) into the air sampling port of the tunnel.

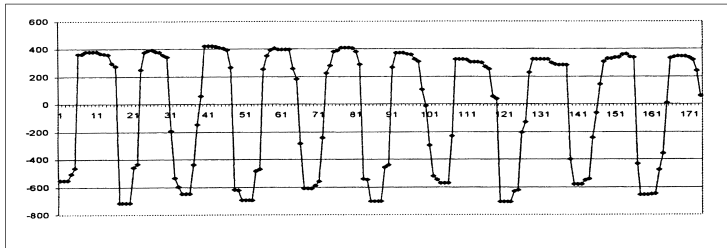
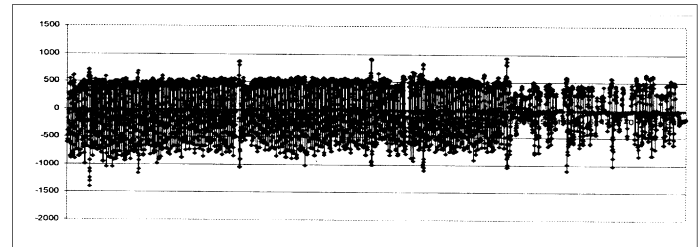
Three experiments (1–3), using a series of 10 pigs in each, and two experiments (4, 5) using a series of 5 pigs in each were performed giving a total of 40 recipient



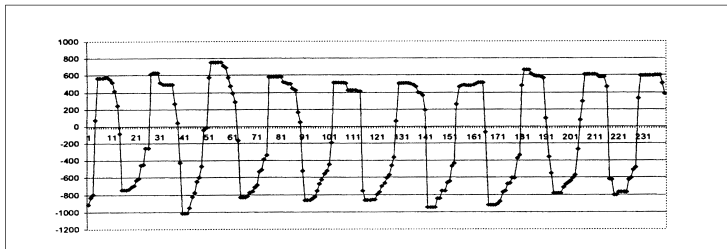
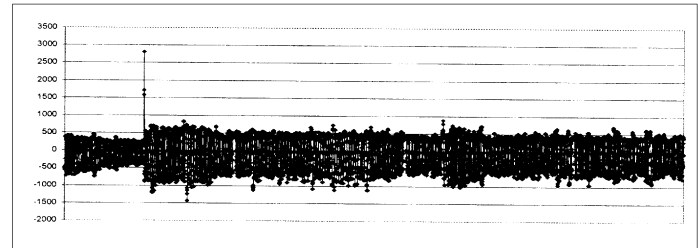
Pig IA6



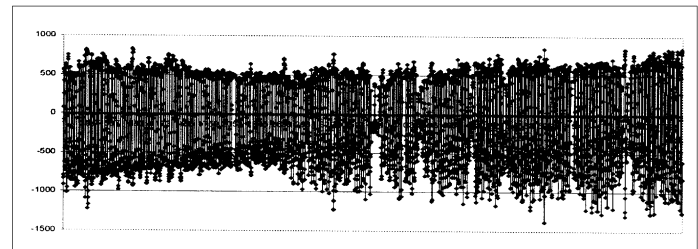
Pig UA8



Pig UA9



Pig UA11



**Fig. 1.** The respiratory patterns of four representative recipient pigs. The left panel shows approx. 10 respiratory cycles while the right panel shows the full 10 min exposure period. The x axes are divided into 80 ms periods and the y axes are in mv, calibrated so that 1 mv corresponds to a flow of 1 ml/s. Inspirations are shown as positive values and expirations as negative values.

Table 1. *Doses of virus to which the pigs in Expt 1 were exposed*

Group	Pig no.	Titre in air TCID <sub>50</sub> /litre	Air inspired litre total	Dose inspired TCID <sub>50</sub>
A	UA6	2.65	158	420
	UA7	3.67	103	378
	UA8	2.60	120	312
	UA9	1.80	120	216
	UA10	1.65	126	208
B	UA11	2.65	150	400
	UA12	0.92	34*	30
	UA13	1.14	114	130
	UA14	—	85	n.d.
	UA15	4.16	129	537

The average dose of detectable virus received by 9 pigs was 293 TCID<sub>50</sub>. The range was from non-detectable (1 pig) to 537. All the pigs were exposed for 10 min, series A at an air-flow of 0.5 m/s and series B at 0.25 m/s. \* Pig UA12 became temporarily disconnected from its mask during the exposure period.

pigs. The concentration of airborne virus flowing through the tunnel was varied by adjusting the airflow 0.25–0.5 m/s. Recipient pigs were exposed individually for periods of 2–10 min. Multiple experiments were done using donor pigs infected with either stock virus 1 or 2.

After exposure, each recipient pig was returned to its cubicle and examined daily for signs of FMD. In order to avoid mechanical transfer of virus the pigs were only handled when blood or nasal swabs were being collected or when they developed signs of FMD. Any clinically affected animal was removed from its cubicle and killed. Blood samples were collected from the pigs in Expts 1 at 14 and 22 d.p.e., and from Expts 2–5 at 7, 10, 14 and 21 d.p.e. Nasal swabs were collected from the pigs in Expt 1 at 14 and 22 d.p.e and from the pigs in Expts 2–5 at 7 d.p.e.

#### 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 exposed to airborne virus. Sampling was done with an all-glass cyclone sampler operating for 2 min at a sampling rate of around 170 litres/min [14].

After the exposure of each recipient pig, an air sample was collected from the exposure tunnel using

a Porton all-glass impinger [25] with 10 ml collecting fluid and operating at 10–13 litres/min for either 2 (Expt 1) or 5 min (Expt 2). In Expts 3–5 a 3-stage liquid impinger [26] with a total of 30 ml collecting fluid and operating at 55 litres/min for 5 min was used instead of the Porton impingers. The collecting fluid employed in the Porton impingers for Expt 1 was MEM–HEPES with antibiotics and 0.1% silicone MS emulsion [14, 15]. The collecting fluid used in the impingers in Expts 2–5 was the same except that the silicone was omitted and BSA added to 0.1%. The port in the tunnel from which air samples were taken was located immediately adjacent to the port at which the recipient pigs were exposed to virus. In Expts 1 and 2, 30 s and 15 s samples of tunnel air, respectively, were collected with the cyclone sampler after five pigs had been exposed. In Expts 3–5 the sampling period was extended to 2 min.

#### Measurement and recording of respiratory function

The respiratory function (rate and volume) of each recipient pig was measured with an ultrasonic phase-shift respiratory flowmeter (BRDL Flowmetrics, Birmingham) [27, 28] located between the tunnel and the exposure mask. The analogue signal from the instrument was converted to digital and recorded on a laptop computer. The signals were recorded every 80 ms and so for a 10 min exposure 7500 readings were obtained. The instrument and ancillary equipment were tested beforehand to ensure the accuracy and linearity of the method. The millivolt readings recorded were converted directly to air flow and registered in ml/s. To obtain respiratory data the recorded readings were transferred to a spreadsheet program (Excel, Microsoft) and a graph program (SlideWrite, Advanced Graphics Software). Inspiration was defined as any positive reading (air flow towards a recipient pig) and expiration as any negative reading (air flow away from a recipient pig). Total inspiration consisted of an integration of amplitude and time of each positive reading and total expiration consisted of an integration of time and amplitude of each negative reading. For a visual display of the data, graphs were produced in both Excel and SlideWrite. Representative respiration graphs of four pigs from Expt 1 are shown in Figure 1. 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

Table 2. *Doses of virus to which the pigs in Expt 2 were exposed*

Group	Pig no.	Titre in air TCID <sub>50</sub> /litre	Air inspired litre total	Dose inspired TCID <sub>50</sub>
A	UC25	1.26	28	35
	UC26	0.62	39	24
	UC27	1.40	35	49
	UC28	0.56	25	14
	UC29	—	39	n.d.
B	UC30	—	159	n.d.
	UC31	—	130	n.d.
	UC32	0.63	89	56
	UC33	1.58	124	196
	UC34	1.40	110	154

The average dose of detectable virus received by 7 pigs was 75 TCID<sub>50</sub>. The range was from non-detectable to 196 TCID<sub>50</sub>. The pigs in series A were exposed for 2 min at an air-flow rate of 0.35 m/s and those in series B for 10 min at 0.25 m/s. n.d., not determined.

uneven flow. These values are depicted in Tables 1–5. 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. The latter was 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 the inoculation of monolayer cultures of primary BTY cells in roller tubes [14, 15, 20]. Tenfold dilution series of collecting fluid samples were made and each dilution inoculated onto five tubes. For the assay of virus in blood and nasal swabs each sample in the dilution series was inoculated onto 3–4 BTY tubes. The specificity of the cytopathic effect observed in cell cultures was confirmed by antigen ELISA [23, 24].

#### Assay for antibodies

Serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to FMDV [29–32].

Table 3. *Doses of virus to which the pigs in Expt 3 were exposed*

Group	Pig no.	Titre in air TCID <sub>50</sub> /litre	Air inspired litre total	Dose inspired TCID <sub>50</sub>
A	UC63	—	141	n.d.
	UC64	—	154	n.d.
	UC65	0.7	109	76
	UC66	—	127	n.d.
	UC67*	0.27	109	29
B	UC68	0.4	133	53
	UC69	—	122	n.d.
	UC70	—	102	n.d.
	UC71	—	134	n.d.
	UC72	—	157	n.d.

The average dose per pig of those receiving measurable amounts of virus (3 pigs) was 53 TCID<sub>50</sub>. The span range from 0 (less than measurable) to 76 TCID<sub>50</sub>. All the pigs were exposed for 10 min at an airflow of 0.25 m/s.

Table 4. *Doses of virus to which the pigs in Expt 4 were exposed*

Group	Pig no.	Titre in air TCID <sub>50</sub> /litre	Air inspired litre total	Dose inspired TCID <sub>50</sub>
A	UD50	0.27	146	40
	UD51	0.43	67	30
	UD52	1.10	111	121
	UD53	1.10	113	123
	UD54	6.88	135	930

The average dose per pig was 250 TCID<sub>50</sub> (range 30–930 TCID<sub>50</sub>). The average without the aberrant value of 930 TCID<sub>50</sub> is only 80 TCID<sub>50</sub>. All the pigs were exposed for 10 min at 0.45 m/s.

Table 5. *Doses of virus to which the pigs in Expt 5 were exposed*

Group	Pig no.	Titre in air TCID <sub>50</sub> /litre	Air inspired litre total	Dose inspired TCID <sub>50</sub>
A	UE64	0.27	100	27
	UE65	2.75	160	440
	UE66	4.35	145	630
	UE67	1.10	105	115
	UE68	4.35	95	415

The average dose per pig was 330 TCID<sub>50</sub> (range 27–630 TCID<sub>50</sub>). All the pigs were exposed 10 min at an airflow of 0.45 m/s.

## RT-PCR

Blood and nasal swab samples were tested for the presence of FMDV viral RNA by the reverse transcription polymerase chain reaction (RT-PCR) method described [33].

## RESULTS

### Airborne virus recovery, respiratory function and exposure doses

The amount of virus in air samples, the concentration of virus in the air, the volumes of air inhaled by the pigs and the total dose to which each pig was exposed are shown in Tables 1–5. The average dose a pig received in each experiment was calculated by: (I) excluding all the pigs which did not receive a measurable dose of virus (negative air sample); (II) adding the measurable amounts of virus received by the other pigs in the experiment; and (III) dividing that sum by the number of those pigs. Therefore, the number of pigs in each experiment on which calculations were based consisted only of the number of pigs which received detectable amounts of virus.

In brief, the data shown in Tables 1–5 can be summarized as follows:

#### Expt 1

9 pigs receiving an average dose of 293 TCID<sub>50</sub>.

#### Expt 2

7 pigs receiving an average dose of 75 TCID<sub>50</sub>.

#### Expt 3

3 pigs receiving an average dose of 53 TCID<sub>50</sub>.

#### Expt 4

5 pigs receiving an average dose of 250 TCID<sub>50</sub>.

#### Expt 5

5 pigs receiving an average dose of 330 TCID<sub>50</sub>.

The respired volume for each pig was calculated as the average inspiration and expiration in order to minimize fluctuations caused by leaks or uneven flow. More details about representative respiratory measurements of four pigs from Expt 1 are shown in Figure 1.

### Air sampling of corridor air

Samples of air from the corridor collected before the exposure of recipient pigs, i.e. just after the donor pigs were placed in the aerosol production chamber were negative for virus in Expts 1 and 4 but positive in

Table 6. Assay of pig sera by liquid-phase-blocking-ELISA (Expt 1)

Pig no.	Antibody titre		
	Prebleed	d.p.e.14	d.p.e.22
UA6	neg	90	181
UA7	neg	181	90
UA8	neg	neg	neg
UA9	neg	n.d.*	
UA10	neg	neg	neg
UA11	neg	128	128
UA12	neg	181	32
UA13	neg	45†	neg
UA14	neg	neg	neg
UA15	neg	90	neg

\* n.d., not done, killed on d.p.e. 5, titre then 64.

† A titre of > 40 is considered positive [37].

Expts 2, 3 and 5. However, the quantity of virus was very low (0.04–0.25 TCID<sub>50</sub> per litre air sampled) compared to that to which recipient pigs were exposed. All of the air samples collected from the corridor after the exposure of recipient pigs were negative.

### Clinical signs, viraemia and seroconversion

The only recipient pig which developed clinical signs of FMD was No. UA9 in Expt 1. At 4 d.p.e. it had painful feet and small vesicles on the snout and on the coronary bands of the feet. It was killed the next day when the clinical signs were severe. Pig UA6 showed decreased activity and reduced appetite at 3–4 d.p.e. However, at 5 d.p.e. its appearance and appetite were normal. Post-mortem examination of pig UA9 revealed vesicular lesions on all four feet, the gingival mucosa, the tongue and snout. No other gross pathological lesions were found. Tissues from this pig were collected into neutral buffered formalin for histopathology and in RNAlater (Ambion) for RT-PCR analysis. The RT-PCR studies together with data on another set of samples snap-frozen and stored at –70 °C for virus titration analysis is described elsewhere [34, 35]. Histopathological examination of haematoxylin and eosin stained sections confirmed the presence of vesicular lesions but no other microscopic lesions were evident. In particular, there was no evidence of any cytopathogenic or inflammatory change in the trachea or lungs. Serum and epithelial tissue samples collected at 5 d.p.e. contained 10<sup>2.2</sup> TCID<sub>50</sub>/ml and 10<sup>5</sup> TCID<sub>50</sub>/g, respectively. A

Table 7. Assay of pig sera by liquid-phase-blocking-ELISA (Expt 5)

Pig no.	Antibody titre		
	Prebleed and d.p.e.7 and d.p.e.10	PED14	PED22
UD64	neg	22	45*
UD65	neg	neg	neg
UE66	neg	neg	neg
UE67	neg	neg	neg
UE68	neg	neg	neg

\* A titre of > 40 is considered positive [37].

serum sample from pig UA9 tested for antibody to FMDV had a titre of 64 at 5 d.p.e.

None of the other 8 recipient pigs in Expt 1 or any of those in Expts 2–5 developed clinical signs of disease. Blood and nasal swabs taken at 14 d.p.e. (Expt 1) and at 7 d.p.e. (Expts 2–5) were negative for FMDV by cell culture and by RT-PCR. Nasal swabs taken at 22 d.p.e. (Expt 1) were negative for FMDV by cell culture. However, serum samples taken at 14 and 22 d.p.e. (Expt 1) showed that 6 pigs, including pig UA6, of the 9 remaining recipient pigs, had antibodies to FMDV (Table 6). Thus, of 9 pigs exposed to a detectable dose of virus (Table 1), 1 developed typical signs of FMDV, 1 was depressed for 2 days and had an increased antibody titre, 5 were subclinically infected and 2 remained normal. Interestingly, the serum antibody titre of pig UA6 had increased at 22 d.p.e., while the other pigs had the same or a lower titre than at 14 d.p.e., indicating that they had experienced an infection of very short duration.

Antibodies were not detected in any of the recipient pigs in Expts 2–4. In Expt 5, a single pig (UE64) had an ELISA titre of 22 at 14 d.p.e. and 45 on day 21 suggesting that this animal might have had a transient infection.

The results can be summarized as follows:

#### Expt 1

9 pigs receiving an average dose of 293 TCID<sub>50</sub>. One pig developed clinical FMD, 6 were subclinically infected and 2 remained normal.

#### Expt 2

7 pigs receiving an average dose of 75 TCID<sub>50</sub>. None was infected.

#### Expt 3

3 pigs receiving an average dose of 53 TCID<sub>50</sub>. None was infected.

#### Expt 4

5 pigs receiving an average dose of 250 TCID<sub>50</sub>. None was infected.

#### Expt 5

5 pigs receiving an average dose of 330 TCID<sub>50</sub>. One pig was subclinically infected.

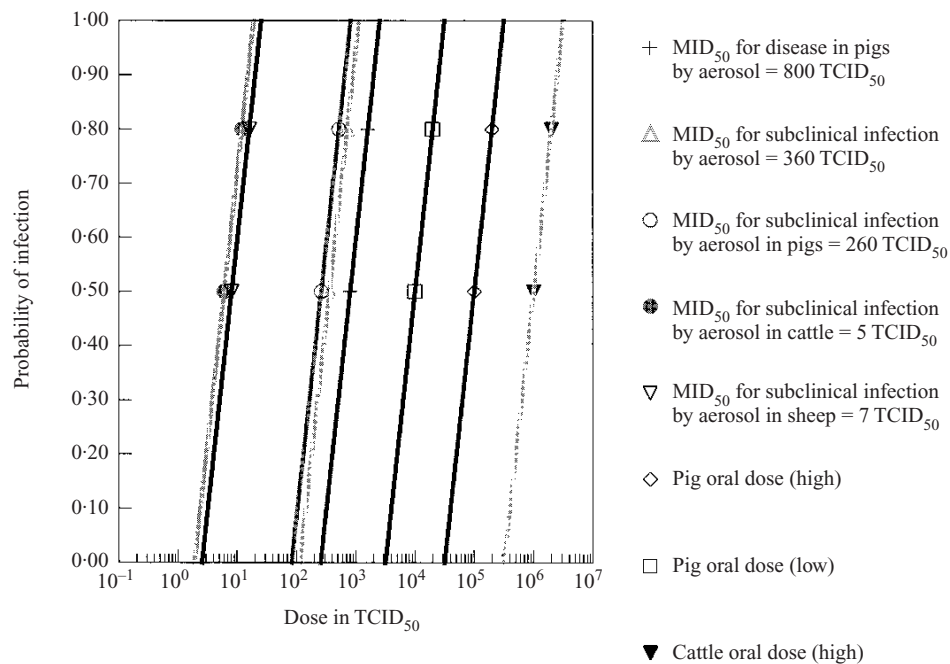
The data presented above can be analysed in several ways. We decided to take Expts 1 and 5 together (inoculum No.1) and Expts 2–4 together (inoculum No.2). Thus of the 14 pigs in Expts 1 and 5 which received an average dose of 306 TCID<sub>50</sub> from donor pigs infected with stock virus 1, 1 pig became clinically infected and 7 were subclinically infected. Therefore the 50% aerosol dose for subclinical infection is around 260 TCID<sub>50</sub> per pig (calculated as  $306/10^{0.5714-0.5}$ ) calculated after Kärber (as described in [36]). Further testing of the two samples with a borderline reaction in the ELISA antibody assay (45), indicated that these two samples had titres of 8 in neutralization test, i.e. were below the  $11 \pm$  cut-off point [37]. When these animals are excluded from the positive results, the calculated aerosol MID<sub>50</sub> (subclinical) is increased to 360 TCID<sub>50</sub> (6 of 14 pigs infected). The average dose of 306 TCID<sub>50</sub> actually administered caused clinical disease in one animal, indicating a 50% disease dose of around 820 TCID<sub>50</sub>. However, since this estimate is based on only one animal the standard error is probably large.

In Expts 2–4 a total of 15 pigs received doses from 53 TCID<sub>50</sub> (3 pigs) to 75 TCID<sub>50</sub> (7 pigs) and 250 TCID<sub>50</sub> (5 pigs) from donor pigs infected with stock 2 virus. However, none of them became infected. Considering that only one pig in Expt 5 became subclinically infected after exposure to an average dose of 330 TCID<sub>50</sub> from stock virus 1, it is not surprising that of the five pigs in Expt 4, which received 250 TCID<sub>50</sub> of stock virus 2, none was infected. Furthermore, the relatively high average dose of 250 TCID<sub>50</sub> in Expt 4 was mainly due to a single sample with a high virus content. This sample was the last in the series and collected after five recipient pigs in the experiment had been exposed to virus. Therefore, the true average exposure dose in Expt 4 was probably around 100 TCID<sub>50</sub>.

## DISCUSSION

The main objective of the study was to determine the minimal infectious dose (MID) of FMDV for pigs when administered as a natural aerosol so that the data could be used to enhance the capability of the





**Fig. 2.** A graph showing the probability of infection on the  $y$  axis related to dose on the  $x$  axis (log scale), the  $MID_{50}$  for aerosol infection of cattle, sheep and pigs (at slightly different estimates) and the doses which will infect cattle and pigs by the oral route.

computer-based model Rimpuff [18]. Rimpuff is an atmospheric model that can predict the risk of airborne FMDV infection for cattle and sheep exposed to a plume of virus but not for pigs since there is a lack of reliable data about the MID of FMDV for that species.

The results obtained show that of the 40 pigs exposed to airborne virus, 6–8 (depending on test cut-off), were infected but only 1 developed typical signs of FMDV. The average amount of virus excreted by donor pigs infected with inoculum 1 (Expts 1 and 5) was higher than those infected by inoculum 2. The converse was expected since inoculum 2 had been passed serially in pigs.

In the calculations used to estimate MID and  $MID_{50}$  (50% MID) values we have included all the pigs which became sub-clinically infected after exposure. We consider that the inclusion of sub-clinically infected pigs in the dose calculations is justified for two reasons. Firstly, sub-clinically infected animals have the potential to transmit infection to other in-contact susceptible species and are therefore of epidemiological significance [38], and secondly, evidence of active FMDV infection, e.g. a rise in antibody, in a domesticated livestock species is, by definition, an outbreak of FMDV [39].

We have concluded from the calculations that

around 300  $TCID_{50}$  is the  $MID_{50}$  for aerosol transmission of FMDV to pigs. There is a probability, however, that virus plumes containing a virus concentration of 100  $TCID_{50}$  might infect some pigs if a large number were exposed, but doses as low as 10–15  $TCID_{50}$ , or less, that consistently infect ruminants are unlikely to infect pigs under natural conditions.

In order to compare the sensitivity of cattle and sheep with that of pigs the MID data available for cattle and sheep were converted to  $MID_{50}$ . The published MIDs for FMDV for sheep and cattle are 10–25  $TCID_{50}$  by natural aerosol [14, 15]. However, the inputs for those calculations were the numbers of clinically affected animals. Re-analysis of data using the Karber equation and including the sub-clinically infected (sero-positive) animals gave values of 5 and 7  $TCID_{50}$  as the  $MID_{50}$  for cattle and sheep, respectively (Fig. 2).

Although low doses, e.g. 10  $TCID_{50}$  or less, can infect ruminants, such doses are unlikely to infect pigs. The reason why cattle and sheep are much more susceptible to infection by airborne FMDV than pigs is not known. It may be due to the presence in the respiratory tract of ruminants of higher concentrations or more effective FMDV receptors. Further work is required to determine whether the MID varies with the strain of FMDV.

The exposure of pigs, cattle and sheep to low doses of FMDV often results in sub-clinical infection. The transient antibody responses found in sub-clinically infected pigs in the present study and the failure to detect virus at 7 and 14 d.p.e. suggest that virus circulation ceased before infection progressed to cause clinical disease [34]. It is probable that this early antibody response was primarily due to IgM [40].

If it is assumed that a dose of 3 times  $MID_{50}$  will produce close to 100% infection then an aerosol dose of 780  $TCID_{50}$  would cause sub-clinical infection in nearly all pigs. Similarly, the dose to induce clinical disease in close to 100% of pigs would be around 2500  $TCID_{50}$  ( $3 \times 820 TCID_{50}$ ). We were unable to test this as it was not possible to produce natural aerosols containing such high doses of FMDV. From a theoretical perspective [36, 41] the exposure of pigs to 1/3 of a  $MID_{50}$ , i.e. around 85–100  $TCID_{50}$  could result in a small number of animals being infected. Those animals could then amplify the virus and transmit it to others either directly or indirectly. However, none of the 15 pigs exposed to 53–250  $TCID_{50}$  (Expts 2–4) became infected, which suggests that there is a threshold level below which infection does not occur, or more likely, is below the susceptibility of the pigs or the detection limit of the *in vitro* assay systems used.

In the present study the methods used to produce and deliver airborne FMDV to pigs were kept as close to the natural situation. The aerosols of virus originated from infected pigs and were delivered to recipient pigs through a tunnel in which the humidity was maintained within a range favourable for airborne virus survival. Recipient pigs were exposed to aerosol through a mask while under light general sedation (minimal dose of Propofol). The volume of air respired by each pig recorded by an ultrasonic phase-shift respiratory flowmeter (BRDL Flowmetrics) which did not require the use of flow-valves and so caused no resistance along the aerosol delivery system. The respiration of the pigs was therefore considered to be 'natural' with the frequency and tidal volume being within expected values, consistent with a light anaesthesia. To reduce the variation in volume of air flow from pig-to-pig due to potential differences in air flow, all data were related to 'respired air volume' which was defined as the average positive value of calculated inspiration and calculated expiration.

The amount airborne virus recovered from the exposure tunnel during sequential sampling showed

wide variation. The reasons for this are not understood but may relate in part to the fluctuation in the quantity of virus exhaled by the donor pigs due to changes in their respiration and/or the kinetics of infection. Also, the manner in which the air samplers were operated and the assay system used were probably close to their limits of detection and so small changes in the amount of virus recovered had a considerable influence on the calculated concentrations of virus in the air. In order to moderate these variables the calculations of MID and  $MID_{50}$  (except the  $MID_{50}$  for clinical disease) were based on estimations of the average dose received by a group of pigs. In this way the potential statistical limitation in establishing the infectious dose for a single pig was changed to group average which is more accurate.

It is not possible to compare our results directly with those reported previously by Terpstra [19] since he used artificially generated aerosols which are known to cause an atypical infection [15, 17]. Also, Terpstra used a mouse assay system for determining the MID. The mouse assay system is much less sensitive than the BTY cell system for the detection of FMDV [11] and so the doses obtained by Terpstra cannot be directly related to those obtained in the present study. Furthermore, the number of animals which might have been infected in Terpstra's experiments could have been under-estimated since all the animals he challenged were killed early after exposure. Thus, our value of approximately 300 BTY  $TCID_{50}$  for the  $MID_{50}$  for pigs cannot be related to the value of 400 mouse  $ID_{50}$  obtained by Terpstra.

The finding that pigs are relatively more resistant to airborne FMDV than are cattle and sheep is in agreement with experimental observations and circumstantial evidence from the field. In the present study it was demonstrated that the transmission of the O<sub>1</sub> Lausanne strain FMDV from infected to susceptible pigs could be prevented if physical barriers were used to prevent infected pigs from making direct physical contact with susceptible pigs and if measures were taken to prevent the mechanical transfer of virus. Similar results were obtained in another study but with an A<sub>5</sub> strain of FMDV (G.O. Denny, unpublished results). Circumstantial evidence from Asia suggests that on occasions when different species of livestock have been at risk of airborne infection through being close to farms containing infected pigs it has been the ruminant species but not the pigs which have been infected (C. Beningo, personal communication).

## ACKNOWLEDGEMENTS

We thank Geoffrey Hutchings, Teli Rendle, Nigel Ferris, Scott Reid and Morag Forsyth for their excellent technical assistance. Natasha Smith and Martin Broomfield are thanked for their assistance with the handling and management of experimental animals. The research was supported by the UK Ministry of Agriculture, Fisheries and Food and the Danish Ministry of Food, Agriculture, and Fisheries.

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