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 (FMD) can be spread by a variety of mechanisms, including, under certain climatic and epidemiological circumstances, by the wind. While the quantities of airborne virus excreted by animals infected with historical strains of the virus have been determined there is relatively little information for contemporary strains and furthermore, the aerosol MID for pigs needs to be more accurately quantified. The objective of the study was to obtain data for the O1 Lausanne (1965), O SKR 2000, O UKG 2001, and C Noville (1973) strains of FMDV to enhance the capability of airborne virus simulation models.

The collection of air samples near pigs infected with these strains has shown that the amount of virus (in TCID50) emitted per pig per 24 hours was: 10^5.8 for O SKR 2000; 10^6.1 for O UKG 2001; 10^6.4 for O Lausanne; and 10^7.6 for C Noville.

The results indicate that the previous estimate of “above” 800 TCID50 as the MID50 for the O1 Lausanne strain was a considerable under-estimate and that the actual dose may be as high as 6000 TCID50. A dose of around 650 TCID50 of the O SKR 2000 strain failed to infect any pigs. Pairs of recipient pigs kept physically separated from donor pigs and exposed to aerosol doses of around 50 TCID50 per minute of the O UKG 2001 strain or 130 TCID50 per minute of the C Noville virus over 24-48 hour periods failed to infect any of eight pigs exposed to O UKG 2001 and only resulted in a transient antibody reaction (subclinical infection) in one out of 8 pigs exposed to C Noville. These results confirm previous findings that pigs, compared to cattle and sheep, are relatively resistant to infection by airborne FMDV.
INTRODUCTION

FMD 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 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 \(^2,12\).

The determination of the biological parameters of the airborne spread of FMD 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 FMD \(^6,9,11-13,16,17,21,24,25,27\). A parameter which has not been quantified in sufficient detail, although the subject of recent preliminary findings \(^1,7\), is the minimal infectious dose 50% (MID\(_{50}\)) of airborne FMD virus needed to infect pigs.

The objective of the present investigation was to expand data for the MID\(_{50}\) of airborne virus using additional strains of FMD virus 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 contemporary strains of FMD virus, the O SKR 2000 and the O UKG 2001 strains (both members of the type O PanAsia group of strains) as well as the historical serotype C Noville (Swiss 73), known to cause excretion of airborne virus at high levels \(^10\).

METHODS

Animals

The pigs were Landrace cross-bred Large White weighing between 20 and 30 kg. Four separate experiments were done. Three “donor” pigs, i.e. animals selected from a group of four inoculated animals as a source of natural aerosols of FMD virus, and eight or ten “recipient” pigs, i.e. animals exposed to airborne FMD virus, were used in each of Experiments 1 and 2. In Experiment 3, a total of 5 pigs were inoculated and then transferred each 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. Experiment 4 was performed identical to experiment 3, although using the C Noville inoculum. Also for this experiment a single pig in one of the groups managed to escape from the cubicle, and thus that box were excluded from the experiment. Therefore, the results from Experiment 4 also 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 and inoculated as described previously \(^1\) with approximately 0.5 ml of stock virus O\(_1\) Lausanne for Expt. 1, stock virus O SKR 2000 for Expt. 2, stock virus O UKG 34/2001
for Expt. 3 and stock virus C Noville (Swiss 73) for exp. 4. All the inocula were diluted 1:10 in MEM-HEPES (Eagle’s Minimal Essential Medium with 20 mM HEPES buffer and x2 antibiotics). Titration of the inocula showed that each animal received around $10^{5.5}$ BTY TCID$_{50}$ of the O Lausanne inoculum, around $10^{5.5}$ TCID$_{50}$ of the O SKR 2000 inoculum or around $10^{7.5}$ TCID$_{50}$ of the O UKG 34/2001 isolate and the C Noville virus.

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 generalised vesicular disease were present (2 or 3 days after inoculation) three pigs (Expt. 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 (Expt. 1 and 2) or for 24 to 48 hours after showing the first vesicular lesions (Expt. 3 and 4).

Recipient pigs were housed singly (Expt. 1 and 2) or in pairs (Expt. 3 and 4) in cubicles constructed within biosecure isolation rooms as described previously 1.

After each recipient pig had been exposed to airborne virus (Expt. 1 and 2) it was returned to its cubicle and examined daily for signs of FMD over a three-week period. For Expt. 3 and 4 the recipient pigs were not exposed in the chamber, instead they were exposed to the virus emitted over a 24 to 48 hour 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 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 (dpe).

**Virus**

The O$_1$ Lausanne Sw/65 strain of FMD virus was used. It had been passed in cattle and then grown in IB-RS-2 cells 4;5. The titre of this stock virus was $10^{6.7}$ TCID$_{50}$ when assayed in primary bovine thyroid (BTY) cells and $10^{5.7}$ TCID$_{50}$ in IB-RS-2 cells. This stock virus was used for Expt. 1 and is the same O$_1$ Lausanne inoculum as used 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 titres of this stock virus were $10^{6.45}$ and $10^{5.7}$ 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 from a pig at Brentwood Abattoir, Essex, UK during the 2001 epidemic in the UK. 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 in aliquots at $-70^\circ$ C. The titres of this stock virus were $10^{8.8}$ and $10^{7.6}$ TCID$_{50}$ per ml in BTY and IB-RS-2 cells, respectively.

The virus used for experiment 4 was original 1st cell culture passage (BTY cells) of field material (Swiss 73) kept frozen for many years. Approximately $10^4$ TCID50 were inoculated into a single pig, and at day 3 severe disease was evident. A virus stock was
prepared from foot epithelial lesions. The titer of this C Noville inoculum was $10^9$ TCID/ml.

**Exposure of pigs to natural aerosols of FMD virus**

The procedures used were modifications of those described previously for both pigs, cattle and sheep $^{1,11,16}$. In brief, three donor pigs were selected at 2 to 3 dpi when they had signs of early generalised FMD and placed in the aerosol production chamber $^{10}$. The chamber was then disinfected on the outside and moved to the other end of the corridor where 2 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 sedated by injection with Propofol as described previously $^1$. 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 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 liter air per kg pig per minute. This estimate was based on the individual measurement of 39 pigs of 20-30 kg of weight $^1$.

The experimental design for Expt. 3 and 4 was different. Recipient pigs, two per cubicle, in a series of 4-5 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 to 48 hours 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 (Expt. 1 and 2) or left in the cubicle (Expt. 3 and 4) and observed daily for signs of FMD. In order to avoid mechanical transfer of virus the pigs were only handled when blood 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 dpe.
Air sampling methods
Cyclone sampling of the animal boxes and May sampling of the exposure cabinet were performed as described previously \(^1\). In Expt. 3 and 4 air samples were also collected from 2 isolation rooms each containing exposed animals. Sampling was done with all-glass cyclone samplers operating for 2 min (Expt. 1 and 2) or 20 min (Expt. 3 and 4) at a sampling rate of around 170 litres/min \(^{16}\).

During the exposure of each pair of recipient pigs (Expt. 1 and 2) an air sample was collected from the aerosol production cabinet using a 3-stage liquid impinger \(^{22}\). In Expt. 3 and 4 two samples were collected from the cabinet with the same sampler when 3 donor pigs taken from each of two isolation rooms were placed in it.

Assay for virus
The infectivity in the collection fluid from air samplers and in blood samples were assayed by inoculation of monolayer cultures of primary bovine thyroid (BTY) cells in roller tubes \(^{26}\). The specificity of the cytopathic effect observed in cell cultures was confirmed by antigen ELISA \(^{15,18,23}\).

Assay for antibodies
Serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to FMD virus \(^{14,19}\). Positive samples were confirmed by virus neutralization test.

RESULTS
Airborne virus recovery and estimated respiration and exposure doses
The average concentration of virus in the air, the average dose inhaled by the pigs and the dose excreted as airborne virus per pig in each experiment are shown in Table 1.

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 hour period per adult pig (NB, calculated as a pig of around 90-100 kg, which equates to three small donor pigs). The excretion of O SKR 2000 averaged \(10^{5.8}\) and the O UKG strain \(10^{6.1}\) TCID\(_{50}\) per 24 hour per pig (90-100kg). The airborne excretion of the C Noville virus was clearly the highest, i.e. \(10^{7.6}\) TCID50 per pig per 24 hours. In Expt. 3 the amount of virus to which recipient pigs were exposed equated to \(10^{5.5}\) TCID\(_{50}\) and in expt. 4 to \(10^{6.5}\) TCID\(_{50}\) per 24 hour per room. This lower challenge dose was due to the continued operation of the ventilation in the rooms which was around 3-5 air-changes per hour.

Clinical signs, viraemia and seroconversion
The only recipient pig which developed clinical FMD was No. UG 77 in Expt. 1. At 4 dpe it was lame and showed vesicles on the snout and on the coronary bands 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.

None of the other 7 recipient pigs in Expt. 1 nor any of those in Expt. 2 or 3 developed signs of disease. Blood samples taken at 7, 10, 14 and 21 dpe (Expt. 1) showed antibodies to FMD virus in 4 out of the remaining 7 recipients, specifically at 10 or 14 dpe. Thus, of 8 pigs exposed to a very high dose of virus (Table 1), one developed
typical signs of FMD and four were subclinically infected. Interestingly, by 21 dpe those pigs were negative for serum antibody, indicating, as seen previously \(^3\), that they had experienced an infection of very short duration.

Antibodies were not detected in any of the recipient pigs in Expt. 2 and 3 (data not shown), except for the excluded single pig (UJ 28) in Expt. 3 which had been in direct contact with the inoculated donor pig.

In expt. 4 a single recipient pig had a transient, weak antibody reaction at 14 dpe. This pig did not show any clinical signs of disease and had no vesicular lesions and thus was subclinically infected.

In all, 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 minute). 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 \(^2\))). The dose to cause clinical disease may be as high as 4000 to 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 minute). None of the pigs developed FMD nor 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 hours which equates to an accumulated dose of more than 70 000 TCID\(_{50}\). None of the pigs developed FMD or detectable antibodies. Thus, when accumulated over a 24 hour period, it seems that the MID\(_{50}\) dose to infect pigs with the UKG isolate may be higher than 70 000 TCID\(_{50}\). Thus, a concentration of around 2500 TCID\(_{50}\) per m\(^3\) (as found in this experiment) is apparently not sufficient to infect pigs with this strain, even when they were exposed for 24 hours or more.

Expt. 4: 8 pigs receiving an average dose of 130 TCID\(_{50}\) per min for at least 24 hours which equates to an accumulated dose of more than 200 000 TCID\(_{50}\). Only one of the pigs developed a transient antibody response, but no clinical disease. Thus, when accumulated over a 24 hour period, it seems that the MID\(_{50}\) dose to infect and cause disease in pigs with the C Noville isolate may be higher than 200 000 TCID\(_{50}\). Thus, a concentration of around 6000 TCID\(_{50}\) per m\(^3\) (as found in this experiment) is apparently only sufficient to subclinically infect a low proportion of pigs with this strain, even when they were exposed for 24 hours 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\textsubscript{1} Lausanne strain,\textsuperscript{1} only one pig in those studies developed clinical disease so more studies were needed with higher challenge doses and with different strains of virus.

The results show that of the 26 pigs exposed to airborne virus in Expt. 1 to 3, four were subclinically infected and only one developed typical signs of FMD. The infected pigs were in the group exposed to the O\textsubscript{1} Lausanne strain (Expt. 1) and calculated to have inspired around 340 TCID\textsubscript{50} per minute for 5 min. This dose is 5 to 10 times greater than in earlier experiments with the same strain when pigs inspired around 30 TCID\textsubscript{50} per minute for 10 min\textsuperscript{1}. Thus, the previously estimated MID\textsubscript{50} value of above 800 TCID\textsubscript{50} may have been a considerable underestimation and the real value could be much higher, perhaps as high as 6000 TCID\textsubscript{50}. Pigs exposed to the O SKR 2000 strain and calculated to have inspired about 130 TCID\textsubscript{50} per minute 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\textsuperscript{5.8} log TCID\textsubscript{50} per 24 hour per pig compared to 10\textsuperscript{6.4} TCID\textsubscript{50} for the O\textsubscript{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\textsuperscript{6.1} TCID\textsubscript{50} per pig per 24 hours, however none of the recipient pigs in cubicles exposed to this strain at a concentration of around 50 TCID\textsubscript{50} per minute i.e., an accumulated dose of approximately 70 000 TCID\textsubscript{50} per pig in a >24 hour period were infected. Furthermore, ext.4 showed that recipient pigs exposed to around 200 000 TCID\textsubscript{50} of C Noville only one of 8 recipient pigs got subclinically infected. This indicates that the respiratory clearance mechanisms for FMDV inhaled by pigs are very efficient and that aerosols of FMDV virus have to be at very high concentrations to infect pigs. In contrast, contact pigs, even after very brief contact, easily got infected.

The findings in the present paper is supported by our previous study on the O1 Lausanne strain\textsuperscript{1}; experimental findings using the A\textsubscript{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 and R.P. Kitching, unpublished results). Also relevant are field observations by veterinarians in the Philippines who have reported that FMD rarely spreads from one pig premises to another when the possibility of direct contact can be excluded (Carolyn Beningo, personal communication).

We conclude from the present and previous findings\textsuperscript{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 to 2000 and 800 to 6000 TCID\textsubscript{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\textsubscript{50}\textsuperscript{11,16}. Therefore, although a pig excrete as much virus as 60 sheep or cattle\textsuperscript{7,8}, 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\textsuperscript{8}, 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 \[8\]. 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.

In this context, the relatively large difference in maximal airborne excretion for the various isolates in pigs, will most likely have a significant influence on the ability to spread to distant cattle and sheep premises.

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 \[20,28\]. 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 minute for 24 hours (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.

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REFERENCES

Ref Type: Report


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* Dose inhaled is estimated per recipient pig (body weight of 25-35 kg) over the period exposed (5 min or 24 hours), while airborne virus excreted is estimated per 90-100 kg pig, i.e. the excretion from 3 donor pigs for 24 hours.