Natural aerosol transmission of Foot-and-Mouth disease in sheep

Isabel Esteves1*, John Gloster2, Eoin Ryan3, Stephanie Durand1 and Soren Alexandersen1,4
1 Pirbright Laboratory, Institute for Animal Health, Ash Road, Woking, Surrey, GU24 0NF, UK
2 Met Office, based at Pirbright Laboratory
3 Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, UK.
4 Current address: Danish Institute for Food and Veterinary Research, Department of Virology, Lindholm, DK-4771 Kalvehave, Denmark
* esteves@bbsrc.ac.uk

Abstract
An important parameter in estimating and predicting foot-and-mouth disease virus (FMDV) airborne spread is the amount of virus released in aerosols from infected animals and the dose needed to infect susceptible species. Such studies have primarily been done using high doses and short-term exposure but here we report an initial experiment designed to assess longer-term exposure of sheep to a low concentration of an FMDV containing aerosol.

We examined the airborne transmissibility of the FMDV isolate between infected “donor” and “recipient” sheep under experimental conditions, over a two week period. The donor and recipient sheep were in same room but in different cubicles in order to avoid any direct or indirect physical contact between animals. Different air samplers were used and samples were collected in the room and in a 610 litre sampling cabinet where the donor sheep was temporarily placed. The infectivity in the collection fluid was assayed by inoculating BTY cells and viral RNA was quantified by real time RT-PCR.

Airborne transmission occurred in two out of three recipient sheep, which although not showing any significant clinical disease developed antibodies against FMDV in blood samples and had infectious virus in probang samples collected between days 23 to 35. Infectivity in air samples was detected 1 and 4 days after the inoculation. The main source of virus detected in the room at day 4 seems to be due to excretion from the infected recipient sheep. The amount of airborne virus recovered from the room and the cabinet at day 1 was $10^{3.48-4.69}$ and $10^{4.11-4.84}$ TCID50 per 24h, respectively. Viral RNA was detected between day 1 and day 4. A peak of RNA copies excreted per 24h was observed in the cabinet at day 3 ($10^{6.24-7.06}$) and in the room at day 4 ($10^{8.83-9.76}$). In summary, even at low dose, airborne transmission occurred after longer-term exposure of sheep to an FMDV containing aerosol. The next step will be to determine the minimal infectious dose by reducing the exposure time of the recipient sheep.

Introduction
Foot-and-mouth disease virus (FMDV) can be spread by a variety of mechanisms including airborne spread (Alexandersen et al., 2003c). An important parameter in estimating and predicting airborne spread is the amount of virus released in aerosols from infected animals and the dose needed to infect susceptible species by the airborne route (Alexandersen and Gloster, 2004). Such studies have primarily been done using short-term exposure but here we report an initial experiment designed to assess longer-term exposure of sheep to a low concentration of an FMDV containing aerosol.

Materials and Methods
Animal inoculation and transmission experiment
We examined the transmissibility of the FMDV O UKG 34/2001 isolate (Alexandersen and Donaldson, 2002b; Alexandersen et al., 2003a) between infected donor and recipient sheep under experimental conditions. Three isolation rooms in a biosecure building containing two cubicles per room were used (Alexandersen, S., Brotherhood, I. and Donaldson, A. I., 2002a; Alexandersen and Donaldson, 2002b). These cubicles prevent any direct or indirect physical contact between animals. The experiment was performed by placing one inoculated sheep (donor sheep) in each cubicle of the three rooms and one recipient sheep in the other cubicle in each of the rooms. One of the donor sheep had its lamb (1-2 weeks of age) in the same cubicle. Donor sheep were inoculated intradermally in the coronary band on the left fore foot (Alexandersen et al., 2003c) with 0.5ml of a suspension of the UKG 34/2001 isolate of FMDV virus from the 2001 UK epidemic passaged once in pigs. The inoculum contained $10^{6.2}$ TCID50/ml when assayed in bovine thyroid cells (BTY) (Snowdon, 1966) and thus each inoculated sheep received $10^{5.9}$ TCID50.

Donor sheep were observed daily for signs of FMD over a two-week period, except for one infected sheep, which was killed on day 7 post infection (p.i.). This sheep belonged to another experiment and the reason why it was killed is explained in a separate presentation (Ryan, E). The recipient sheep
were only handled on day 14, 18, 23, 25 and 35 when blood samples were collected for testing for the presence of antibodies to FMD virus by ELISA (Hamblin et al., 1986). Oesophageal/pharyngeal (probang) samples were also collected at day 23, 25, 28 and 35 to test for the presence of virus. We also examined the virus excretion of lambs infected by direct contact in a separate isolation room. This experiment was performed by infecting three ewes as above and having their 1-2 weeks old lambs in direct contact in the same box. The experimental detail of this experiment is described in a separate presentation (Ryan, E).

Air sampling methods
On days 0 (before inoculation), 1, 2, 3, 4 and 7 p.i. air samples were collected in one of the rooms containing one donor and one recipient sheep. The following air sampling equipment was used: Porton impinger (May and Harper, 1957), 3 stage liquid impinger (May, 1966), Cyclone sampler (Errington and Powell, 1969) as described previously (Alexandersen, S., Brotherhood, I. and Donaldson, A. I., 2002a; Alexandersen and Donaldson, 2002b; Alexandersen et al., 2002c; Alexandersen et al., 2003b) and a newly developed “AB” sampler (patent pending). Air samples were also taken with a Porton sampler connected in series after the AB sampler.

One donor sheep was also put temporarily in a 610 litre sampling cabinet on days 1, 2, 3 and 4 for air sampling (Alexandersen et al., 2002c, Donaldson and Ferris, 1980); the May, Porton and AB sampler were used. Air sampling from lambs were also taken in the cabinet at day 1, 2, 3 and 4.

Air sample assays
The infectivity in the collection fluid (except for the AB sampler) was assayed by inoculating BTY cells (Alexandersen, S., Brotherhood, I. and Donaldson, A. I., 2002a; Snowdon, 1966). Viral RNA in all air samples was quantified by real time RT-PCR as described previously (Alexandersen et al., 2002c; Alexandersen et al., 2003b, Reid et al., 2001; Reid et al., 2002; Reid et al., 2004). The results were expressed as the quantity of virus released in 24h (log TCID_{50}/24h). These values take into account the sample volume, the flow rate, the sampling time and the animal mean breathing volume (method A). The conventional way of calculating airborne excretion of FMD virus was also included for comparison to previous papers. This calculation included the sample volume and the sampling time (method B).

Results
Infection
All donor sheep presented typical clinical signs of FMD in sheep (lesions and fever) within 1-2 days after inoculation. At that time, viral RNA was also detected in serum and nasal swabs. Airborne transmission occurred in two out of three recipient sheep which although not showing any significant clinical disease developed antibodies against FMD virus in blood samples collected at days 14-35 p.i.. The same two sheep were also positive for virus by testing of probang samples taken on days 23-35 p.i.. Only one of the virus-positive recipient sheep had fever 4 to 7 days after the inoculation of the donor sheep.

Airborne virus recovery
The amount of airborne virus recovered from the room and the cabinet at day 1 was 10^{3.48-4.69} and 10^{4.11-4.84} TCID_{50} per 24h, respectively (Table 1). In order to make the titres comparable between the room, which was ventilated (ten air changes per hour), and the cabinet, which was not ventilated, the quantity of virus recovered in room samples were adjusted upwards with 1 log10 unit (10-fold). Day 4 data from the cabinet samples suggest that the main source of virus detected in the room likely was due to excretion from the recipient sheep (Table 1). Viral RNA was detected between day 1 and day 4. A peak of RNA copies excreted per 24h was observed in the room at day 4 (10^{8.83-9.76}) and in the cabinet at day 3 (10^{8.24-7.06}). The concentration of FMD virus aerosol in the room at the peak were approximately 0.02 TCID_{50} per litre of air as estimated from the Cyclone sample which means that the recipient sheep were exposed to an accumulated dose by the airborne route of around 300 TCID_{50} in a 24 hour period. As mentioned, this dose resulted in 2 out of 3 recipient sheep becoming (subclinically) infected.

Table 1- Estimated release of airborne virus in 24h (method A/method B)^a

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>Infectivity (log TCID_{50})</th>
<th>Genome (log RNA copies)</th>
<th>Room</th>
<th>Cabinet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room c,d</td>
<td>Room d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.48 / 4.69</td>
<td>4.11 / 4.84</td>
<td>7.11 / 7.85</td>
<td>5.69 / 6.41</td>
</tr>
<tr>
<td>2</td>
<td>Not detected</td>
<td>3.27 / 3.99</td>
<td>8.31 / 9.56</td>
<td>5.68 / 6.47</td>
</tr>
<tr>
<td>3</td>
<td>Not detected</td>
<td>Not detected</td>
<td>7.85 / 8.53</td>
<td>6.24 / 7.06</td>
</tr>
<tr>
<td>4</td>
<td>3.48 / 4.69</td>
<td>Not detected</td>
<td>8.83 / 9.76</td>
<td>5.57 / 6.08</td>
</tr>
<tr>
<td>7</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>
a Method A: calculation that take into account the sample volume, the flow rate, the sampling time and the animal mean breathing volume; Method B: calculation that take into account the sample volume and the sampling time.
b Mean of all positive samples
c Cyclone sampler
d 1 log10 has been added to compensate for the ventilation
e May sampler
f No AB samples were taken in the cabinet

Virus infectivity was also observed at day 2 in air samples taken from lambs in the cabinet (table 2). The amount of airborne virus recovered per lamb was $10^{3.33-4.51}$ TCID$_{50}$ per 24h. Viral RNA was detected between day 1 and day 4. A peak of RNA copies excreted per 24h per lamb was observed at day 4 ($10^{6.78-7.49}$).

**Table 2- Estimated release of airborne virus per lamb in 24h** (method A/method B)

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>Infectivity (log TCID$_{50}$)</th>
<th>Genome (log RNA copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not detected</td>
<td>5.49 / 6.14</td>
</tr>
<tr>
<td>2</td>
<td>3.33 / 4.51</td>
<td>6.06 / 7.32</td>
</tr>
<tr>
<td>3</td>
<td>Not detected</td>
<td>6.77 / 7.16</td>
</tr>
<tr>
<td>4</td>
<td>Not detected</td>
<td>6.78 / 7.49</td>
</tr>
</tbody>
</table>

a Method A: calculation that take into account the sample volume, the flow rate, the sampling time and the animal mean breathing volume; Method B: calculation that take into account the sample volume and the sampling time.
b Mean of all positive samples
c Cyclone sampler
d 1 log10 has been added to compensate for the ventilation
e May sampler
f No AB samples were taken in the cabinet

The mean ratio between genome and infectivity for samples that were positive with both techniques was 300 +/- 30. This value is comparable to the ratio found in e.g. serum samples during the viraemic phase (Alexandersen et al., 2003b).

In contrast to what was observed for infectivity, RNA copies were detected with all air samplers (Tables 1 and 2). The sampler that detected more RNA copies was the Cyclone ($10^{8.85}$), followed by the AB and the Porton samplers in series ($10^{8.55}$), the AB sampler ($10^{8.17}$), the Porton ($10^{6.93}$) and finally the May sampler ($10^{4.83}$).

Discussion
In the present study, FMD transmission by longer-term exposure of sheep to a low concentration of an FMDV containing natural aerosol was examined. Two out of three recipient sheep developed subclinical disease. The concentration of FMD virus aerosol in the room at the peak was approximately 0.02 TCID$_{50}$ per litre of air as estimated from the Cyclone sample. Assuming that an adult sheep inhales about 15 l/min (Alexandersen et al., 2002c; Donaldson et al., 2001) the accumulated dose received by the recipient sheep was around 300TCID$_{50}$ in a 24h period. In a previous experiment in which sheep were exposed for 2 h to airborne virus from pigs, almost all (3 out of 4) of the airborne infected sheep developed FMD clinical signs. But in this study the FMD virus concentration was much higher (about 1.5 TCID$_{50}$ per litre of air) and animals received an estimated accumulated dose of 4000TCID$_{50}$ (Aggarwal et al., 2002, Donaldson et al., 2001).

A previous experiment has shown that a dose of 10TCID$_{50}$ was enough to infect sheep (Gibson and Donaldson, 1986). In the present study, the recipient sheep are likely to have inhaled an infectious dose within approximately 1 hour. So the next step will be to determine the minimal infectious dose by reducing the exposure time.

The recipient sheep excreted airborne virus 4 days after infection of the inoculated sheep. In a previous experiment where sheep were infected by direct contact, excretion of airborne virus was maximal after 48h. The airborne accumulated dose received by the recipient sheep in that experiment was higher than in the present study and the sheep were continuously and closely confined so transmission to the in-contact could have occurred by several different routes explaining the speed of the transmission. This was confirmed with the lamb experiment in which virus excretion was observed 48h after infection by direct contact.

The quantity of virus excreted by the airborne infected sheep was the same as the needle infected sheep. Despite this same virus quantity, the airborne infected sheep did not develop any clinical symptoms except fever in contrast to what it was observed for the needle infected sheep. Another important finding was the quantity of airborne virus excreted by lambs which was as high as virus excreted by adult sheep. This is potentially of major epidemiological significance if subclinically infected sheep and lambs truly excrete as much virus as clinically affected sheep.
In the present study different air sampler were compared. Virus infectivity was detected with the Cyclone and May sampler. These samplers collected larger volume of air and thus may have greater sensitivity than the other sampler. In contrast, all samplers allowed genome detection and the best air samplers were the Cyclone and the AB sampler. A Porton sampler added in series after the AB sampler increased the quantity of viral RNA recovered. Generally, genome detection is more sensitive than infectivity detection and the ratio values between these two techniques are similar to what it was observed previously in serum samples during the viraemic phase (Alexandersen et al., 2003b). This observation may be explained by the highest sensitivity of the PCR technique. The detection of genome without infectivity may be due to the violent impact of air into the impinger fluid that may destroy virus infectivity. It may also be due to the variability of the BTY cells infection assay that depends on the sensitivity of cells to virus infection. Also the manners in which the air samplers were operated and the assay system used were probably close to their limits of detection.

Conclusions
- Even at low concentration, airborne transmission occurred after longer-term exposure of sheep to an FMDV containing aerosol.
- Airborne virus excreted by lambs was as high as excreted by adult sheep.
- These data will increase the accuracy of predictive models that simulate airborne spread.

Recommendations
- Detailed studies of airborne transmission of FMD under controlled conditions should be intensified in order to improve the accuracy of simulation models

Acknowledgments
The research was supported by the Department for Environment, Food and Rural Affairs (DEFRA), UK. We thank Iain Brotherhood for his involvement in development of the AB air sampler.

References


