Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001

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The profiles of virus production and excretion have been established for sheep experimentally infected with the UK 2001 strain of foot-and-mouth disease (FMD) virus by inoculation and by direct and intensive contact. Virus replicated rapidly in the inoculated sheep, from which a peak infectivity of airborne virus of $10^{4.3}$ TCID$_{50}$ per sheep per 24 h was recovered. Around 24 h later, contact-infected sheep excreted airborne virus maximally. Similar amounts of airborne virus were recovered from cattle. The excretion of virus by the sheep under these conditions fell into three phases. First, a highly infectious period of around 7–8 days. Second, a period of 1–3 days soon afterwards when trace amounts of viral RNA were recovered in nasal and rectal swabs. Third, at 4 weeks after exposure, the demonstration, by tests on oesophageal–pharyngeal samples, that 50% of the sheep were carriers. The implications of the results and the variable role that sheep may play in the epidemiology of FMD are discussed.

Introduction

Foot-and-mouth disease (FMD) is a virus disease of domesticated and wild ruminants and pigs. FMD virus (FMDV) is a member of the genus Aphthovirus within the family Picornaviridae (Belsham, 1993) and has a significant degree of variability – seven serotypes and more than 60 subtypes exist (Haydon et al., 2001; Knowles et al., 2001). The isolate used in the present studies belongs to serotype O and has specifically been characterized as a ‘PanAsian strain’ (Knowles et al., 2001). FMD is feared by farmers and veterinary authorities because of its highly contagious nature and the drastic measures required to eradicate the infection. As a consequence, FMD is the major disease constraint to international trade in livestock and animal products.

The contagious nature of FMD is a reflection of a number of factors, including the wide host-range of the virus, the amount of infectivity excreted by affected animals, the low doses required to initiate infection and the many routes of infection. FMD is most often spread when infected and susceptible animals are brought into contact. Various mechanisms of transmission are possible in such circumstances – between ruminant animals, the most common route is by the inhalation of infectious droplets and droplet nuclei originating mainly in the breath of the infected animals (Sellers et al., 1971; Sellers, 1971). The next most frequent mechanism of spread is by the feeding of contaminated animal products, e.g. milk and meat. Infection may also be spread by mechanical means, for example when animals come into contact with the 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, but is essentially uncontrollable and can be dramatic (Anonymous, 1969; Donaldson et al., 1982).

Sheep played a major role in the 2001 UK FMD epidemic and it was important to obtain virological and aerobiological data for that species infected with the UK strain of virus. Infection of pigs has been the subject of a separate study (Alexandersen & Donaldson, 2002). The current studies were undertaken with the objectives of (i) generating virus and viral RNA response curves for sheep infected with the O UK 2001 strain of FMDV and (ii) determining the amount of airborne virus excreted by infected sheep and cattle for incorporation into predictive models that simulate airborne spread.

Methods

Animals. Ten female, Dorset cross-bred sheep, weighing around 30 kg, were used. The sheep were shorn of their fleeces and placed in a single room in a biosecure animal building. Six ‘inoculated’ sheep, i.e. animals selected at random from the group, were infected by injection as described below. Four ‘contact’ sheep were kept in the same room
throughout the experiment. The humidity was kept above 60% and the ventilation in the animal room was reduced to three air changes per hour. Thus, the experimental design created optimal conditions for virus transmission, simulating intensive, indoor conditions. In a subsequent experiment, two heifers were used (Friesian Holstein breed, around 150 kg body weight).

The inoculated sheep were injected intradermally/subdermally in the coronary band of a left fore foot (Burrowes, 1968) with 0.5 ml of a stock virus (for details, see below). The inoculum was diluted 1:10 in MEM–HEPES (Eagle’s minimal essential medium with 20 mM HEPES buffer and antibiotics). Each animal received around 10^{5.5} TCID_{50}. The two heifers were inoculated with the same virus by subdermo-lingual injection (Henderson, 1949, 1952).

Each day until 10 days post-inoculation (p.i.), the animals were examined clinically and rectal temperatures were recorded. Blood samples and nasal and rectal swabs were taken daily for the first 2 weeks after inoculation (only on days 0–3 for the cattle). The samples were transferred immediately to the laboratory; swabs were processed immediately and stored as mentioned below while blood samples were kept at 4 °C for 16–24 h and the serum separated. An aliquot of each serum was diluted 1:1 with Total nucleic acid lysis buffer (Roche) and stored at −80 °C until subjected to subsequent analysis by real-time reverse transcriptase–polymerase chain reaction (RT–PCR) and subsequent analysis by real-time 5′-nuclease RT–PCR. The rest of the serum was frozen immediately and stored at −80 °C. Swabs were taken in duplicate; one swab was placed in 2 ml maintenance medium and stored at −80 °C and the other was placed in 1 ml TRIzol (Life Technologies) and stored at −80 °C. Oesophageal–pharyngeal (‘pro-bang’) samples were taken from the sheep at 28 days after exposure. These samples were shaken with an equal volume of buffer (pH 7.2–7.4) and stored frozen at −80 °C until analysed. The sheep were killed at 28 days p.i. and the cattle at 3 days p.i.

**Virus.** The virus suspension was prepared from foot epithelium obtained from a pig during the 2001 UK FMD epidemic. The virus isolate is denoted FMDV O UKG 34/2001. A 10% (w/v) suspension was made in MEM–HEPES and stored at 0.5 ml aliquots at −80 °C. The titres of this stock virus were 10^{8.8} and 10^{6.6} TCID_{50}/ml in primary bovine thyroid (BTY) cells (Snowdon, 1966) and in IB-RS-2 cells (De Castro, 1964; De Castro & Pisani, 1964), respectively.

**Measurement of aerosol excretion of FMDV from sheep and cattle infected with UKG 34/2001.** Samples of the air in the rooms containing infected sheep or cattle were collected on days 1 and 2 p.i. (sheep) and days 1 and 3 p.i. (cattle). These days were selected on the basis of earlier findings (Donaldson et al., 1970; Sellers & Parker, 1969). In addition, air samples were collected from a series of three pairs of inoculated sheep that were selected on the same days and placed for about 15 min in a 610 l cabinet (Donaldson & Ferris, 1980). Multiple air samples were collected. The relative humidity was kept high (above 60%) and therefore suitable for the survival of airborne FMDV (Donaldson, 1972).

**Air sampling methods.** Air samples from the animal rooms were collected with an all-glass cyclone sampler operated for 20 min at a flow rate of around 170 l/min (Gibson & Donaldson, 1986).

The air in the cabinet containing each pair of sheep was sampled with a three-stage liquid impinger (May, 1966) containing a total of 30 ml collecting fluid. The sampler was operated at 55 l/min for 5 min. The collecting fluid employed in the impingers was MEM–HEPES with antibiotics and 0.1% (w/v) BSA (Donaldson et al., 1987; Gibson & Donaldson, 1986).

The concentration of virus per litre of air was determined by endpoint titration of the collecting fluid of the particular air-sampler, multiplied by the volume of the collecting fluid and the flow of the sampler. The amount of infectivity recovered was expressed as the total amount of airborne FMDV in TCID_{50} per sheep or heifer per 24 h.

**Assay for virus.** The infectivity of the collection fluid in air samplers and in selected blood samples and swabs was determined by assay in monolayer cultures of BTY cells in roller tubes (Donaldson et al., 1987; Gibson & Donaldson, 1986). Tenfold dilution series of collecting fluid samples were made and each dilution was inoculated onto five tubes. Titrates were calculated using the Karber equation according to Lennette (1964). The specificity of any CPE was confirmed by antigen ELISA (Ferris et al., 1988; Ferris & Dawson, 1988; Hamblin et al., 1984; Roeder & Le Blanc Smith, 1987).

**Quantitative RT–PCR.** A quantitative RT–PCR method was used to determine the amount of FMDV RNA in extracts of total nucleic acid from blood and swab samples. Conversion to TCID_{50} equivalents/ml was made according to Alexandersen et al. (2001) and Oleksiewicz et al. (2001). The assay method was similar to that used previously (Alexandersen et al., 2001; Oleksiewicz et al., 2001) except that the primers and the probe (patent pending) were altered so that the assay was able to detect all isolates of FMDV (Reid et al., 2001). The specific conditions used will be published in detail elsewhere (Reid et al., 2002).

All samples were stored in lysis buffer or in TRIzol until subjected to automated total nucleic acid extraction in a MagnaPure LC robot (Roche). All extractions involved 0.1 ml sample and the nucleic acid was finally eluted in a volume of 0.1 ml. Thus, the nucleic acid was more dilute than in earlier investigations (Alexandersen et al., 2001; Oleksiewicz et al., 2001), however, the extraction had several advantages over the manual method. Firstly, the extraction was very consistent and gave highly reproducible results. Furthermore, because the samples were more dilute and of a greater purity than in previous work, only a single dilution was assayed (i.e. an amount corresponding to 0.003 ml initial sample in a single RT–PCR). Fifty serum samples from the sheep on days 0–4 were tested in duplicate. Eighty-five per cent of these duplicates fell within a single C_{50} (C is ‘cycle threshold’, the first cycle where a sample can be detected as positive). Fifteen per cent of the samples showed more variability. The higher variability in this small percentage of samples was probably caused by faulty or inaccurate manual pipetting and, subsequently, the system was changed to include a robotic arrangement of the RT reaction and PCR. With this procedure of automated nucleic acid extraction and robotic RT and PCR, the variability was consistently within a single C_{50} for duplicate samples.

All estimations included standard reactions using samples with a known content of FMDV (as determined by virus titration in cell culture) and all quantifications were based on a comparison with standard curves based on tenfold dilution series of cell culture supernatant at 40 h after infection with FMDV (infectious titre determined by virus titration) as described in detail previously (Alexandersen et al., 2001; Oleksiewicz et al., 2001). The method is influenced minimally by sample type and has a sensitivity (still in the quantitative range) of approximately 0.1 TCID_{50}/ml of either serum, cell culture or swab fluid. In most figures, the levels of FMDV RNA are given by the expression of 50−C_{50} in order for samples with a higher content to be shown easily on the graphs. For figures showing the relationship between real-time RT–PCR signal and virus infectivity in cell culture, the RT–PCR values are given as (50−C_{50})×2.3 in order to correlate the two dimensions, of which one had a log_{10} scale (RT–PCR) and the other a log_{10} scale (cell-culture titration).

**Assay for antibodies.** Serum samples were tested for the presence of antibodies to FMDV by an ELISA using homologous virus passaged in cell culture as described previously (Ferris, 1987; Ferris et al., 1990; Hamblin et al., 1986a, b, 1987).
Results

Airborne virus recovery and estimated aerosol excretion and exposure doses

The amounts of airborne virus recovered from the animal room containing inoculated and contact sheep, the cabinet containing inoculated sheep and the animal room containing inoculated cattle are shown in Tables 1 and 2. In brief, the data show that sheep and cattle excreted up to 10%–90% TCID₅₀ per animal per 24 h.

In order to make the titres comparable between the room, which was ventilated (reduced to three air changes per hour), and the cabinet, which was not ventilated, an amount of 10⁻¹⁻⁵ TCID₅₀ was added to the quantity of virus recovered in room samples. Taken together, the samples collected from the cabinet containing inoculated sheep and the animal room containing all 10 sheep (both inoculated and contacts) indicated that peak virus excretion by inoculated sheep occurred at 1 day p.i., while the source of virus on the subsequent day most likely originated from the contact sheep.

Clinical signs, virus load in serum and swabs and seroconversion

Inoculated sheep developed local vesicular lesions (unruptured) at the injection sites as well as increased temperature of one or more additional feet (generalization) by 1–2 days p.i. and all six sheep showed mild clinical signs of lameness on days 2–5. Fever, defined as a temperature above 40 °C, began after

Table 1. Amounts of airborne FMDV recovered from the cabinet containing a pair of inoculated sheep or from the animal room containing both inoculated and contact-infected sheep

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of sampling</th>
<th>Sheep</th>
<th>Virus recovered in sample (log₁₀ TCID₅₀/ml)</th>
<th>Concentration of airborne virus (TCID₅₀/l)</th>
<th>Total airborne virus (TCID₅₀)</th>
<th>Estimated release of airborne virus in 24 h (log₁₀ TCID₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabinet samples (55 l/min for 5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 day p.i.</td>
<td>UI 97, UJ 03</td>
<td>0⁻⁸</td>
<td>0⁻⁹</td>
<td>190</td>
<td>4⁻⁷ for two sheep, 4⁻⁴ per sheep</td>
</tr>
<tr>
<td>2</td>
<td>1 day p.i.</td>
<td>UJ 01, UJ 00</td>
<td>0⁻⁴</td>
<td>0⁻²⁷</td>
<td>75</td>
<td>4⁻³ for two sheep, 4⁻⁰³ per sheep</td>
</tr>
<tr>
<td>3</td>
<td>2 days p.i.</td>
<td>UI 97, UJ 03</td>
<td>Negligible</td>
<td></td>
<td>No airborne virus detected</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 days p.i.</td>
<td>UI 96, UI 99</td>
<td>Negligible</td>
<td></td>
<td>No airborne virus detected</td>
<td></td>
</tr>
<tr>
<td>Room samples (170 l/min for 20 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 day p.i.</td>
<td>All 10 sheep</td>
<td>Negligible</td>
<td></td>
<td>No airborne virus detected (below detection limit)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 days p.i.*</td>
<td>All 10 sheep</td>
<td>1⁻⁸⁺</td>
<td>0⁻²</td>
<td>63¹</td>
<td>4⁻⁷</td>
</tr>
</tbody>
</table>

* Collected at day 2 p.i. for inoculated sheep, which is probably day 1 post-infection for the contacts.
† An amount of 10⁻⁷ TCID₅₀ was recovered from the room containing six inoculated and four contact-infected sheep. The data from the cabinet samples suggest that the main source of virus at this time (day 2 p.i.) was the four contact-infected sheep. This equates to 10⁻¹⁻⁵ TCID₅₀ per (contact) sheep, equivalent to about 10⁻⁷ TCID₅₀ per 24 h per contact sheep at day 1 post-exposure (multiplied by three, i.e. an addition of 10⁻⁷ TCID₅₀ to account for reduction by ventilation; see text).

Table 2. Amounts of airborne FMDV recovered from infected cattle

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of sampling</th>
<th>Concentration of virus (log₁₀ TCID₅₀/ml)</th>
<th>Total airborne virus (TCID₅₀)</th>
<th>Estimated total release of airborne virus in 24 h (log₁₀ TCID₅₀)</th>
<th>Mean release per heifer (log₁₀ TCID₅₀ per 24 h per animal)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 day p.i.</td>
<td>1⁻⁴</td>
<td>252</td>
<td>4⁻³</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 day p.i.</td>
<td>1⁻⁰</td>
<td>100</td>
<td>3⁻⁹</td>
<td>4⁻⁻³⁻⁵</td>
</tr>
<tr>
<td>3</td>
<td>3 days p.i.</td>
<td>1⁻⁴</td>
<td>252</td>
<td>4⁻³</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3 days p.i.</td>
<td>&lt; 0⁻⁵</td>
<td>&lt; 30</td>
<td>&lt; 3⁻³</td>
<td>4⁻²⁻⁴</td>
</tr>
</tbody>
</table>

* An addition of 10⁻⁷ TCID₅₀ was made to each value to account for ventilation; see text.
1–2 days and lasted up to 5 days. The mean time for the development of fever was at day 2 in the inoculated group. Among the contact group, only a single sheep showed signs (increased temperature of a foot) at day 1 after inoculation of the donor group. At days 2–6, more sheep from the contact group showed signs of lameness so that, on day 6, all four sheep had shown clinical signs of disease. Fever lasted from 1 to 3 days in this group. The mean time when fever was first detected was at day 4 in this contact group (after inoculation of the injected sheep). The time after exposure that lesions, increased temperature or clinical signs of disease (lameness) were first observed was on average 1–4 days for the inoculated group and around 3 days for the contact group.

The cattle showed only mild signs of disease and had a minor temperature increase. At 1 day p.i., both heifers had an excess nasal secretion. At 2 days p.i., both heifers were lame on one foot, although lesions were not obvious. At 3 days p.i., the two heifers were killed. Examination revealed severe lesions on the tongue (ruptured vesicles), but only mild generalized lesions, in the form of small, ruptured vesicles on the dental pad. In addition, small unruptured vesicles were found on one or two feet. No rumenal lesions were observed.

The results obtained for the quantities of virus in the inoculated sheep showed that viraemia (defined as detection of FMDV genomic material in serum samples by real-time RT–PCR) was detectable from 1 day p.i. and reached peak values of around 10^6–7 TCID_{50} equivalents/ml at 2–3 days p.i. All of the inoculated sheep ceased to exhibit viraemia by 5–8 days p.i. All of the contact-infected sheep ceased to have viraemia by day 8 (Fig. 1). Selected serum samples were also tested by virus titration in cell culture (data not shown) and, as before, strong correlation with the RT–PCR assay was found.
FMD virus loads and excretion in sheep and cattle

Fig. 4. FMDV genomes in nasal swab samples from sheep. The mean as well as the \( \text{so} \) in each group (inoculated sheep, \( n = 6 \); contact sheep, \( n = 4 \)) are shown in relation to the number of days after the start of the experiment. Signals are expressed as 50\( -\text{Ct} \) and the corresponding values for TCID\(_{50}\) equivalents/ml are indicated for certain levels. The corresponding levels of airborne virus excreted from the sheep are also indicated as the amount of airborne virus excreted per sheep per hour, as derived from the data in Table 1.

Fig. 5. FMDV genomes in rectal swab samples. The mean and \( \text{so} \) in each group (inoculated sheep, \( n = 6 \); contact sheep, \( n = 4 \)) are shown in relation to the number of days after the start of the experiment. See legend to Fig. 4 for further details.

(Alexandersen et al., 2001); however, none of the serum samples contained detectable levels of infectious FMDV after day 7. However, low levels of viraemia (genomic material) were found by RT–PCR in some day 9–11 samples. None of the 10 sheep had detectable viraemia on days 12, 13 or 28 either by virus isolation or by RT–PCR (Fig. 1). However, the signals for the day 9–11 serum samples corresponded to very low FMDV genome levels, most likely below the detection limit of cell culture. The results were repeatable, so it was concluded that low-level viraemia (defined as presence of FMDV RNA) can be seen for a few days in infected animals after the initial peak viraemia is cleared by the developing antibody response. In fact, similar patterns were seen with nasal and rectal swab samples (see below).

Peak viraemia in both groups coincided with body temperatures above 40 °C, which, as mentioned earlier, peaked at days 2 and 4, respectively, for the inoculated and contact sheep.

The correlation between the infectivity of swab samples and TaqMan RT–PCR was compared by testing 40 nasal swabs (taken at days 3, 4, 5 and 8 p.i.) and 30 rectal swabs (taken at days 3, 4 and 8 p.i.) by both methods. A strong correlation was found with samples containing more than \( 10^{9} \) TCID\(_{50}\) virus/ml; however, samples with little or no detectable live virus were often positive in the RT–PCR and so this assay appears to deliver the most accurate quantification of virus load, even though the virus detected may not necessarily be infectious (Figs 2 and 3). Nevertheless, infectivity and RT–PCR reactivity were strongly correlated for the day 3, 4 and 5 samples. On day 8, all of the samples were negative for infectivity (below detection limit), but several had a low reaction by RT–PCR. This indicated that there was a strong correlation between the assays and that samples that were weakly positive by RT–PCR but negative in cell culture were just below the detection limit of that particular assay. For the rectal swabs, a moderately strong linear correlation between

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\begin{align*}
10^{9} \text{TCID}_{50}/\text{ml} \\
10^{6} \text{TCID}_{50}/\text{ml} \\
10^{3} \text{TCID}_{50}/\text{ml} \\
1 \text{TCID}_{50}/\text{ml}
\end{align*}
\]

\[
\begin{align*}
\text{Arbitrary units (50-Ct)}
\end{align*}
\]

\[
\begin{align*}
7 & 14 & 21 & 28
\end{align*}
\]

Time after injection of inoculated sheep (days)
infectivity and RT–PCR signal was found; however, the infectivity of these samples was clearly much lower than for the nasal swabs, even for samples having a comparable signal in RT–PCR. This suggested that the virus infectivity was reduced by alimentary passage or, alternatively, that the rectal swabs contained some material that reduced the sensitivity of the cell-culture system. Day 8 samples were negative by virus isolation, but a few samples were weakly positive in the RT–PCR, while day 3 and 4 samples were positive for infectivity at a low level (up to about $10^2$ TCID$_{50}$) but had a stronger signal by RT–PCR.

The virus loads found in the nasal and rectal swabs are shown in Figs 4 and 5. The levels are generally higher in the nasal swabs, with a mean of about $10^4$ TCID$_{50}$ equivalents or about $10^3$ TCID$_{50}$ equivalents or less. FMDV RNA peaked in the nasal swabs around days 3–4 and in the rectal swabs at day 5.

The temporal development of antibodies is shown in Fig. 6. Antibodies were detected in inoculated sheep from 4 or 5 days p.i. and reached high levels by 7 days p.i. Contact sheep were antibody-positive from day 6 or 7 and reached high levels by day 8 or 9. The development of measurable levels of antibodies correlated well with a sharp decrease in viraemia.

Samples from the two cattle were also examined (data not shown in detail). Briefly, nasal swabs were positive on days 1–3 p.i. at a level corresponding to $10^{16}$–$10^{9}$ TCID$_{50}$ virus/ml. This value is similar to that found for the day 3 and 4 sheep samples and in probang or in serum samples from heifers at 1–3 days p.i. (data not shown).

**Carriers**

At 4 weeks after exposure, three of the six inoculated sheep were carriers (two virus-positive and an additional sheep positive by RT–PCR) and, of the four contact sheep, two were carriers (one virus-positive and this one and another sheep positive by RT–PCR). However, at 4 weeks p.i., all the serum samples and all the nasal swabs were negative, but one of 10 sheep had a positive rectal swab by RT–PCR. This particular animal was a carrier both by virus isolation and by RT–PCR. Interestingly, when the results of the RT–PCR analysis of the 10 sheep were divided into two groups of five sheep (five carriers and five non-carriers), it was seen that the mean number of days detected positive was higher in the carrier group for both nasal and rectal swabs and, furthermore, that the mean peak levels in these swabs were also higher. The differences detected in the serum samples were less pronounced; however, the mean peak viraemia was higher by approximately 2 C$_t$ (indicating that the peak viraemia of carrier animals may be around fourfold higher than that of non-carrier animals).

**Discussion**

A critical determinant of an FMD epidemic is the ability of the virus infection to spread within and between premises under field conditions. The rate and extent of spread will be determined by the inherent properties of the virus, the amount of virus excreted, the degree of direct and indirect contact, the susceptibility of the species affected and the specific agricultural and environmental conditions. An analysis of the first 10 days of the 1967–68 UK epidemic, when spread was attributed to the transmission of virus by the wind, resulted in widespread transmission (Anonymous, 1969; Donaldson, 1979, 1986; Donaldson et al., 1982; Gloster et al., 1982). Such widespread transmission is most likely to occur when there has been airborne spread of virus from pigs to ruminants or, alternatively, when there has been extensive contact between infected and susceptible animals under conditions of dense stocking. Recent analyses indicate that airborne spread over a distance of more than 6–20 km is unlikely for most contemporary FMDV isolates (Donaldson et al., 2001; S. Alexandersen, R. P. Kitching, J. H. Sorensen, T. Mikkelsen, J. Gloster and A. I. Donaldson, unpublished data). However, certain FMDV isolates may be excreted at very high levels and may have caused airborne spread beyond 20 km (S. Alexandersen, Z. Zhang and A. I. Donaldson, unpublished data). The species infected at source is important, since the quantity of airborne virus emitted by sheep and cattle is much lower than that from pigs. Consequently, sheep and cattle are much less likely to be the source of airborne spread than are pigs (Donaldson et al., 1970, 2001; Donaldson, 1979; Sellers & Parker, 1969; Sorensen et al., 2000). Another factor that may result in widespread transmission is when infected animals are moved through markets as part of extensive trading networks, as was seen with sheep during the 2001 UK epidemic. Under these conditions, spread may be rapid, distant and extensive. Furthermore, the effect may be further amplified if the animals are housed, since this will raise the aerial concentration of FMDV and increase the probability, and potentially the load, of infection. In contrast, transmission by direct or indirect
exposed for 2 h to airborne virus from pigs, an incubation experiment with the same strain of virus, in which sheep were infected by the inhalation of highly infectious amounts of airborne virus within 24 h of inoculation and that the inoculated sheep were excreting airborne virus maximally under the experimental conditions selected. The high dose of virus to which both the inoculated and in-contact sheep were exposed was probably the main factor that determined the speed of transmission. Both categories of sheep were continuously and closely confined, so transmission to the in-contact sheep could have occurred by several different routes, e.g. by inhalation, by ingestion or by intra-ocular or percutaneous infection. Considering that the inoculated sheep were excreting large amounts of airborne virus within 24 h of inoculation and that sheep are very susceptible to infection by the respiratory route (Gibson & Donaldson, 1986), it is most likely that the contact sheep were infected by the inhalation of highly infectious droplets and droplet nuclei.

The incubation period in FMD depends on the species, the strain of virus, the route of infection and, especially, the dose of infection. In the present experiment, conducted under intensive indoor conditions, the incubation period for the inoculated sheep was 24–48 h. However, in a different experiment with the same strain of virus, in which sheep were exposed for 2 h to airborne virus from pigs, an incubation period of 6–8 days was observed (Aggarwal et al., 2002). In that experiment, the challenge dose would have been lower, and this was probably the major factor that determined the rate of spread through the groups of sheep. In the field, the dose of exposure would be influenced by several factors. Management activities resulting in the congregation of sheep, such as lambing, shearing, deworming, clinical inspection etc., would increase the challenge dose by the aeroenic route, especially if the animals were indoors, but would also increase the probability of transmission by additional routes. For example, personnel handling the sheep could easily become contaminated by virus in nasal fluid, saliva, vesicular fluid, milk or faeces and transfer it mechanically. The result would be shorter incubation periods in individual sheep and an increased rate of transmission through the flock. By contrast, incubation periods would be longer and the rate of transmission slower among sheep held outdoors under extensive conditions. It could be hypothesized that in some circumstances the level of infection might decline with time and be self-limiting. There is evidence to support this hypothesis both during an epidemic of FMD (Mackay et al., 1996) and from experimental studies using conditions resembling less-intensive, outdoor conditions with a much lower effective contact rate (Hughes et al., 2002).

The results obtained from the recovery of virus in samples of air and from nasal and rectal swabs showed that the excretion patterns of individual sheep could be subdivided into three phases: first, an initial, highly infectious period lasting for around 7–8 days; second, shortly afterwards, a period lasting for 1–3 days when trace amounts of virus RNA were detected in swabs from the respiratory and alimentary tracts as well as in serum samples; and then third, at 4 weeks, the demonstration that 50% of the sheep were persistently infected, i.e. ‘carriers’.

The role of sheep in the epidemiology of FMD results mainly from the first of these phases, since this is when sheep are most infectious. It is of particular epidemiological importance that the emission of airborne virus by sheep occurs from 1 or 2 days before clinical disease is likely to be apparent. Even then, the proportion of sheep with obvious clinical signs may be small (Donaldson & Sellers, 2000). The significance of the second phase of excretion is not clear, but is almost certainly of much less importance in terms of transmission. However, this second phase may possibly be involved in the development of carrier animals or possibly in the generation of variant virus. The third phase, when a proportion of sheep may become carriers, is variable. Around 45% of experimentally infected sheep have been shown to be carriers at 8 weeks and 25% after 12 weeks (Burrows, 1968). The only evidence that carrier sheep may have played a role in the epidemiology of the disease is circumstantial, and this occurred 9 months after infection (E. Stougaard, personal communication, 1983).

The peak amounts of airborne virus recovered from the sheep and cattle in this study were similar to those obtained with other strains of FMDV (Donaldson et al., 1970; Sellers et al., 1970; Sellers & Parker, 1969). Airborne virus was detected on only one of two days of sampling from sheep but on both days 1 and 3 p.i. sampled from cattle. By contrast, other strains of virus were recovered from sheep for up to 48 h longer, although, in those studies, the pattern of very early excretion from sheep was also evident (Donaldson et al., 1970; Sellers et al., 1970; Sellers & Parker, 1969). The recovery, in earlier studies, of amounts of infectivity over a longer period may have been because the sampling devices collected larger volumes of air and thus had greater sensitivity than our system. The peak amounts of virus recovered from sheep and cattle infected with the UK 2001 strain were only around one-sixtieth of that recovered from infected pigs (Alexandersen & Donaldson, 2002). This difference between ruminants and pigs is considerably less than has been found for some other strains of FMDV (Donaldson et al., 1970; Sellers, 1971; Sellers & Parker, 1969; S. Alexandersen, Z. Zhang and A. I. Donaldson, unpublished data).

The concentration of airborne virus in the room containing the sheep was equivalent to $10^{-7}$ TCID$_{50}$/l. Assuming that an adult sheep inspires about 15 l/min (Donaldson et al., 2001), the dose received by the contact sheep was approximately $10^{6}$ TCID$_{50}$ per sheep over a 24 h period. With this concentration of virus in the air, the sheep are likely to have inhaled an infectious dose within approximately 5 min (Donaldson et al., 2001; Gibson & Donaldson, 1986).

The total amount of airborne FMDV emitted from an infected pig farm is the product of the number of affected
animals, determined by careful clinical inspection, multiplied by the excretion values for the particular virus. The risk of airborne spread from the farm can then be assessed using an atmospheric dispersion model and inputting the emission data together with data about the climatic conditions and local topography to simulate plumes of airborne virus (Donaldson et al., 2001; Sorensen et al., 2001). The same can be done with premises containing cattle, although the quantity of airborne virus excreted will be much lower. For sheep premises, it would be more difficult to make accurate estimates of virus emission due to the cryptic nature of FMD in that species. Also, for sheep kept under extensive conditions of husbandry, the amounts of virus excreted at a given time are likely to be less than intensively stocked animals due to the slower progression of the disease in the flock.

Peak viraemia in both the inoculated and contact groups correlated well with the pyrexic responses, which peaked at days 2 and 4, respectively, for the inoculated and contact sheep. The virus load found in the swab samples indicated that the nasal tract, especially, contained significant amounts of virus, while rectal swab samples, although often positive by RT–PCR, were usually negative for or very low in tests for infectivity. Interestingly, as reported previously (Alexandersen et al., 2001), our quantitative RT–PCR assay was proportional to infectivity on samples taken soon after infection, i.e. up to about day 5 after exposure, and therefore before antibody complexes would have been present. The correlation was changed at later stages, presumably because the virus was bound to antibody and not detected in infectivity assays but still gave a positive reaction by RT–PCR. However, it is obvious that the correlation of the two methods on rectal swabs was different from the correlation on nasal swabs and indicated a low infectivity of the rectal swab samples.

The clinical signs in the sheep infected with the UK 2001 strain were typical of FMD in that species; however, the signs in infected cattle were mild and accompanied by a minor pyrexic response. In another experiment with the UKG strain were typical of FMD in that species; however, the signs in infected cattle, although the quantity of airborne virus excreted will be much lower. For sheep premises, it would be more difficult to make accurate estimates of virus emission due to the cryptic nature of FMD in that species. Also, for sheep kept under extensive conditions of husbandry, the amounts of virus excreted at a given time are likely to be less than intensively stocked animals due to the slower progression of the disease in the flock.

We thank Tellervo Rendle, Linda Turner and Geoff Pero for excellent technical assistance. Luke Fitzpatrick, Nigel Tallon, Darren Nunney and Malcolm Turner are thanked for their assistance with the handling and management of experimental animals. The research was supported by the Department for Environment, Food and Rural Affairs (DEFRA), UK.

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Received 19 October 2001; Accepted 26 March 2002