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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SUMMARY
Foot-and-mouth disease virus (FMDV) can be spread by a variety of mechanisms. The objective of the current study was to obtain aerosol excretion data for the O UK 2001 isolate in sheep and in cattle and to measure the time course of virus load (infectivity and viral RNA) in nasal swabs, rectal swabs and serum to formulate a viral load framework for assessment of transmission risks. Oesophageal-pharyngeal (probang) samples were collected from the sheep at 28 days after exposure to establish whether any of them became persistently infected.

Virus replicated rapidly in inoculated sheep from which a peak infectivity of airborne virus of $10^{4.3}$ TCID$_{50}$ per sheep per 24 hours was recovered. Around 24 hours later contact-infected sheep excreted airborne virus maximally, also around $10^{4.3}$ TCID$_{50}$ per sheep. The peaks of airborne excretion of the inoculated and contact sheep were 24 hours apart, lasted for 24 hours and then fell to below detection limits. Similar peak amounts of airborne virus were recovered from cattle, however, they maintained this level of excretion for about 3 days. The excretion of virus by the sheep fell into four phases. Firstly, a period of high excretion of airborne virus as described above. Secondly, a highly infectious period of 5-7 days, when excretions (nasal swabs and rectal swabs as well as serum) had significant levels of infectivity. Thirdly, a period of a few days (1-3 days) just after the infectious period when low amounts of viral RNA were recovered in nasal and rectal swabs as well as in serum. Fourthly, at 4 weeks when oesophageal-pharyngeal samples showed that 50% of the sheep were carriers.

This data provides a basis for developing a more comprehensive picture of the various transmission risks from livestock, especially sheep, at various stages of the infectious process.

INTRODUCTION
FMD is a viral disease of domesticated and wild ruminants and pigs characterised by the development of vesicles in and around the mouth and on the feet. FMD virus is a member of the *Aphthovirus* genus within the *Picornaviridae* family. FMD is feared by farmers and veterinary authorities because of its highly contagious nature, its ability to cause persistent infection in ruminants (carriers) and the difficulties inherent in eradicating the virus following outbreaks. Even vaccinated animals may become carriers when exposed
to live virus. 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 and infection may also be spread by mechanical means, for example via animal contact with virus on the surfaces of transport vehicles, milking machines or on the hands of animal attendants. An additional mechanism is the spread of FMD virus by the wind.

The objective of the present investigation was to study the aerosol excretion from sheep infected by the FMDV type O UK 2001 and to provide a quantitative framework for a more detailed analysis of transmission risks from sheep in particular and to a lesser extent from cattle.

METHODS

Animals
Ten female 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 of the coronary band. Four “contact” sheep were kept in the same room throughout the experiment. In a subsequent experiment two heifers were used (Holsteins).

The Inoculated sheep received 0.5 ml of FMDV O UKG 2001 inoculated intradermally/subdermally in the coronary band of a left fore foot. Titration of the inoculum showed that each animal had received around 10^7.5 TCID50. The two heifers were inoculated with the same virus by subdermo-lingual injection.

The animals were examined clinically each day for signs of FMD. Rectal temperatures were recorded daily until 10 days after inoculation (dpi). Blood samples (serum tubes) and nasal and rectal swabs were taken daily for the first 2 weeks after inoculation (only for day 0 to day 3 for the cattle). The blood samples were immediately transported to the laboratory, kept at 4°C for 16-24 hours and the serum separated. An aliquot of serum from each sample were immediately diluted 1:1 with a commercial RNA stabilizer solution (Roche Lysis Solution) and stored at room temperature until nucleic acid extraction and subsequent analysis by real time 5'-nuclease RT-PCR (to be described in detail elsewhere). The rest of the serum was immediately frozen and stored at –80°C. Swabs were taken in duplicate, one swab was placed in 2 ml of maintenance medium and stored at –80°C while the other one was placed in 1 ml of TRIzol (Life Technologies, Paisley, UK) and stored at –80°C. Probang samples were taken from the sheep at 28 days after exposure. These samples were shaken with a buffer and stored frozen at –80°C until analyzed.

The sheep were killed at 28 and the cattle at 3 days post exposure (dpe).

Virus
The virus used 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 –80°C. The titres of this stock virus were 10^8.8 and 10^7.6 TCID50 per ml in BTY and IB-RS-2 cells, respectively. Each inoculated sheep received approximately 10^7.5 TCID50 (BTY).
Measurement of aerosol excretion of FMDV from sheep and cattle infected with UKG 2001

Samples of the air in the room were collected with a cyclone sampler on the first and second day (sheep) and first and third day (cattle) after inoculation. In addition, a series of pairs of sheep were selected on the same days and placed in a 610 litre cabinet 10 and multiple air samples collected with a May sampler. The inside of the cabinet and the walls and ceiling of the room were sprayed with water before commencing the air sampling to ensure that the relative humidity was high and therefore suitable for the survival of airborne virus 10. After measurement, the sheep were returned to the box.

Air sampling methods

Air samples collected by Cyclone sampling as well as by May sampling at the exposure cabinet were performed as described previously 1.

The peak of virus excreted was expressed as the total amount of airborne TCID50 per sheep/ or heifer per 24 hours (as measured at 1 and 2 dpi (sheep) and 1 and 3 dpi (cattle).

Assay for virus

The infectivity in the collection fluid from air samplers and in other samples were assayed by inoculation of monolayer cultures of primary bovine thyroid (BTY) cells in roller tubes 25. The specificity of the cytopathic effect observed in cell cultures was confirmed by antigen ELISA 15,17,22.

Quantitative RT-PCR.

Quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to determine the amount of FMDV RNA in extracts of total nucleic acid from blood and swab samples. The assay method was similar to that used previously 2,21 to quantitate the amounts of FMDV RNA in tissues from pigs infected with the O1 Lausanne virus. The method used in the current studies involved an almost identical protocol, however, the primers and the probe (patent pending) were changed so that the assay was able to detect all serotypes of FMDV (Reid and others, This meeting!). The specific conditions used will be published in detail elsewhere. All extractions involved 0.100 ml of sample and the nucleic acid were finally eluted in a volume of 0.1 ml. Thus, the nucleic acid was more dilute than in earlier investigations 2,21, however, the extraction method 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 much higher purity than our previously extractions, only a single dilution was assayed (i.e. an amount corresponding to 0.003 ml of initial sample in a single RT-PCR).

All estimations included standard reactions using samples with a known content of FMDV (as determined by virus titration in cell culture), and furthermore all quantitations were based on a comparison with standard curves based on dilution series of infected cell culture supernatant as described in detail previously 2,21. The method is influenced minimally by sample type.

Assay for antibodies

Serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to FMD virus 14,18.
RESULTS

Airborne virus recovery and estimated aerosol excretion and exposure doses
The amount of virus in air samples are summarized in Table 1 (sheep) and Table 2 (cattle).

In brief, the data shown can be summarized as follows:
Excretion of airborne virus was calculated as the average excretion from infected sheep at 1 and 2 dpi after inoculation and from infected cattle at 1 and 3 dpi. Thus, excretion of FMDV O UKG was up to $10^{4.3}$ TCID50 per 24 hour per sheep (weighing about 30-40 kg) and up to $10^{4.3}$ TCID50 per heifer (150 kg).

Therefore the amounts of airborne virus emitted by the two species were very similar, although the heifers were probably around 150 kg and the sheep were only around 30-40 kg. However, the data for the sheep may indicate that peak aerosol excretion lasts for only a single day, while in cattle significant amounts of virus could be detected for 3 days.

Clinical signs, virus load in serum and swabs, and seroconversion
Among the inoculated sheep, 4 animals showed signs of local vesicular lesions (unruptured) at the injection sites as well as increased temperature of one or more additional feet (generalization) by 1 dpi. One sheep had local lesions on day one and generalisation to several feet by day 2. Fever, defined as a temperature above 40°C, lasted from 1-2 days and up to 5 days and was detected in 2 sheep at day 1, 6 sheep at day 2, 4 sheep at day 3, 3 sheep at day 4 and a single sheep on 5 dpi. The average time for development of fever was at day 2 in the inoculated group. At day 2 one or two sheep from the contact group showed signs of lameness while on day 3 a third sheep had increased temperature of a foot and became lame on day 4. Fever (temperature above 40°C) lasted from 1 to 3 days in this group, one animal having fever at day 3 only, one on days 3-5, one on day 4 and the last sheep on day 5 only (after inoculation of the injected sheep). The average time for fever was at day 4 in this contact group. The first time that lesions, increased temperature or clinical signs of disease were 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 a minor temperature increase. At 3 days after inoculation the 2 heifers were killed. Examination showed severe local lesions on the tongue (ruptured vesicles) but only mild additional lesions, in the form of small, ruptured vesicles on the dental pad. In addition, one animal had 3 feet and the other animal had 2 feet with ruptured, small vesicles.

Viraemia in the inoculated sheep was detectable from day 1 and reached peak values of around $10^{4.5}$ TCID50/ml at 2 to 3 dpi. All of the inoculated sheep ceased to have a viraemia by days 5-8. Among the contact animals a single sheep had a very low level of viraemia on day 1 and this animal and one more had a slightly increased level at day 2, however, high levels could not be detected in the contacts before day 3 and peaked, again at around $10^{4.5}$ TCID50/ml, on day 4. All of the contact sheep ceased to have a viraemia by day 8. However, in both groups, but most pronounced in the inoculated group, low levels of FMDV RNA were detected in sera between day 9 and 11. None of the 10 sheep
had detectable viraemia at day 12, 13 or 28. However, the signals in the RT-PCR on 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 and could be seen in
both serum and swab samples.

Peak viraemias in both groups correlated strongly with elevated body temperatures above
40° C, which peaked at day 2 and at day 4 for the inoculated and the contact sheep,
respectively.

Initially the correlation between the virus titres of swab samples on BTY cell cultures was
compared to TaqMan RT-PCR by testing 40 nasal swabs (taken at 3, 4, 5 and 8 dpi).
Correlation was obvious in samples with more than 10^2.0 TCID of virus per ml, however,
samples with little or no detectable live virus were often positive in the RT-PCR and this
assay appears to deliver the most accurate quantitation of viral load, even though the
virus genome detected may not necessarily be infectious. Nevertheless, infectivity and
RT-PCR reactivity were strongly correlated for the day 3, 4 and 5 samples. On day 8 all
samples were negative for infectivity (below detection limit) but several had a low
reaction in RT-PCR. This indicated that the correlation between the assays was strong
and that samples being weak positive in RT-PCR but negative in cell culture are just
below the detection limit of that particular assay. For the rectal swabs a linear correlation
between infectivity and RT-PCR signal was moderately strong, however, the infectivity
of these samples was clearly much lower than the nasal swabs, even for samples having a
comparable signal in RT-PCR. This suggested, that the alimentary passage of the virus
had partially inactivated its infectivity, or alternatively, that the fecal swabs contain some
material reducing the sensitivity of the cell culture system.

Samples from the two cattle were also examined. Nasal swabs were positive on day 1-3
after infection at a level corresponding to 10^4 to 10^5 TCID50 of virus per ml. This value
fits well with the findings for the day 3 and 4 sheep samples and is at a level similar to
that found in probang samples or in serum from the same heifers at day 1-3 after infection
(data not shown).

Antibodies could be detected in inoculated sheep from day 4 and reached high levels by
day 7. Contact sheep were positive from day 6 and reached high levels by day 8.

**Carriers** The 10 sheep were tested for persistent infection at 4 weeks after exposure.
Three sheep were positive (virus isolated from OP-fluid and positive in RT-PCR) while 2
sheep had a low reaction in the RT-PCR but were negative by virus isolation. Thus, by
virus isolation 3 sheep were definitively carriers, and the more sensitive technique of RT-
PCR indicated that a total of 5 sheep had detectable levels of FMDV RNA in probang
samples taken at 4 weeks after inoculation or exposure. Interestingly, all the serum
samples and all the nasal swabs were negative at his time point, however, one out of 10
sheep had a rectal swab positive by RT-PCR at 4 weeks. This particular animal was
positive by both virus isolation and RT-PCR on the OP-fluid and thus was a carrier.
Interestingly, dividing the results of the RT-PCR analysis of the 10 sheep into two groups
of 5 sheep (5 carriers and 5 non-carriers) indicate, that the average number of days
detected positive were higher in the carrier group for both nasal and rectal swabs and
furthermore, that the average peak levels in these swabs also were higher for the carriers.
The differences between these two groups in regard to the levels in the serum were
similar although much less pronounced. Average viraemia was only slightly longer in the carriers, however, the average peak viraemia was increased by approximately 4-fold.

**DISCUSSION**

The experiments described in the current study confirmed that sheep excrete airborne FMDV at maximal levels early in the infection (approximately one day after inoculation and for contact sheep the day afterwards). The maximum amount of virus excreted for the UKG 2001 FMDV corresponds to around $10^{4.3}$ TCID50 in a 24 hour period per sheep (approximately 30 kg). However, peak excretion apparently only lasts for 1 day. The aerosol excretion from infected cattle was similar, i.e. a maximum of $10^{4.3}$ TCID50 in a 24 hour period per heifer (approximately 150 kg), however, in cattle excretion continued at high level for another 2 days. The maximum level of $10^{4.3}$ TCID50 in a 24 hour period is significantly less than what is excreted from pigs infected with this isolate of FMDV. In pigs, excretion up to $10^{6.1}$ TCID50 in a 24 hour period per 90-100 kg pig has been described for this particular isolate, equivalent to an aerosol excretion from pigs approximately 60-fold higher than for sheep and cattle.

Investigations during the UK 2001 epidemic (R. P. Kitching, S Alexandersen and others, unpublished results) have shown that the disease progressed slowly in sheep and that evidence from the field may indicate, that only about 5% or less of the sheep in a flock were infected after several weeks, while in pig herds and cattle herds up to 40-50% of the animals had disease and were excreting FMDV at the same time. The total aerosol excretion from an affected pig farm can be estimated by thorough clinical investigations and calculated on the basis of the excretion values for the UKG 2001 FMDV. The same could be done with cattle premises – although excretion is much lower. For sheep, it is more difficult to make estimates of the maximum excretion levels by sheep flocks due to the cryptic nature of FMD in that species, but the amounts are likely to be very low due to the slow progression of the disease and the short, sharp aerosol excretion period.

This relatively low level of airborne excretion from sheep and cattle confirm previous work suggesting that these species only play a minor role in airborne spread between farms. However, the experiments also showed, that although unlikely to be involved in distance transmission, sheep may easily cause aerosol transmission under local conditions especially when high density housing conditions is used.

For the sheep the peak aerosol excretion apparently only lasted a single day (then fell to levels below our detection limit). This will of course have an impact on the ability to transmit disease. Thus, if an infected animal is not in relatively close contact with other sheep at that particular day, risk of aerosol transmission is greatly reduced. However, as the day of peak excretion is very early in the infection, and before any clinical signs can be noticed, it is very difficult to control this mode of spread in sheep.

In order to explain the variable nature of FMDV transmission in sheep, we propose a dual mechanism to be part of the explanation for the variable spread of FMDV in sheep. An amount of virus responsible for infection may alternatively to coming from airborne virus, come from excretions and be internalized by close direct or indirect contact to infected sheep, as an amount of infectious virus in the period from 3 to 7 days after inoculation or contact of $10^{3.0}-10^{4.0}$ TCID50 may equal the amount of nasal fluid being
taken up by a single swab. However, this amount may need to somehow enter by the respiratory/aerosol route instead of the oral route, in order to be sufficient to cause infection. However, it may be possible, that nasal fluid as well as saliva or perhaps OP-fluid, which all are produced and drooled in large amounts during acute infection, can cause infection if directly or indirectly deposited onto traumatized skin. Nevertheless, as the rectal swabs contained significantly less infectious virus it is considered unlikely to be a major vehicle of spread during an epidemic, however, feces can not be excluded as a potential risk considering the large amounts produced and the finding of, albeit low levels, live virus. Taken together, the findings indicate, that sheep excrete airborne FMDV very early in the infectious process, under the conditions described here the day after inoculation or approximately one day after contact exposure, furthermore, aerosol excretion is only measurable on a single day. Thus, efficient spread of the disease will only occur when contact among excreters and non-infected sheep are widespread and close and are likely to be enhanced by housing, i.e. by maximizing the concentration of aerosolized virus. Rather high amounts of virus infectivity is found in the nasal swabs even at times where airborne excretion has stopped. However, it seems possible, that in situations with occasional close contact, sheep may be infected by close physical contact to infected sheep, most likely in the period from 2-7 days after infection. If spread by indirect means (indirect contact) is to be considered during this period we expect it to be of a relatively low risk unless the contact involve physical handling of a susceptible animal, resulting in exposure to damaged skin. From day 8 and later, we did not isolate any live virus (below detection limit) although low levels of virus RNA could be detected in a number of samples from day 8 to 14. On day 28 virus was isolated from the OP-fluid of 3 sheep and these three samples as well as two additional samples were positive in RT-PCR.

The results on viral loads in the sheep indicated that viraemia were detectable from day 1 and reached peak values of around $10^{4.5}$ TCID50/ml at day 2 to 3 after direct inoculation. Of the contact animals a single sheep had a very low level of viraemia on day 1 and this animal and one more had a slightly increased level at day 2, however, high levels could not be detected in the contacts before day 3 and peaked, again around $10^{4.5}$ TCID50/ml, at day 4. Thus, the level of aerosol excretion can not be correlated to the viraemia levels, apparently the aerosol excretion peaks before viraemia while the data suggest that the nasal excretion (swabs) may peak after the viraemia. In both groups, but most pronounced in the inoculated group, positive reactions could be found using the RT-PCR. It should be mentioned, that the signals on the day 9-11 serum samples corresponded to very low FMDV genome levels, most likely below the detection limit of cell culture. We conclude that a low level viraemia (copies of FMDV RNA) can be seen in infected animals after the first peak is cleared by the antibody reaction. Similar minor peaks could also be observed in the swabs. The mechanisms and potential importance is currently unknown.

The virus load found in the nasal and rectal swabs indicated that especially the nasal tract contained significant amounts of virus while the rectal swabs albeit often being positive by RT-PCR, often were negative or very low regarding infectivity. Interestingly, as we have suggested before the exact correlation of signal in our quantitative RT-PCR assay is only directly proportional to samples taken in early infection, i.e. up to about day 5 after exposure, when the host reaction, including antibody being produced, diminish infectivity with a comparable slower fall in RT-PCR reactivity. However, evidently the
correlation of the two methods on rectal swabs indicated a more intense decrease in infectivity in such samples.

The development of antibodies at days 4 and 6 in inoculated and contact sheep, respectively, fits well with the observed sharp decrease in viraemia. However, it appears, that the decrease caused by antibodies is more pronounced in the blood (serum) than observed in for instance the nasal swabs. A reduced decrease in virus load despite development of antibodies has previously been suggested for epithelial lesions in pigs ².

As mentioned above, virus isolation showed that 3 sheep were definitively carriers, and the more sensitive technique of RT-PCR indicated that a total of 5 sheep had detectable levels of FMDV RNA in probang samples taken at 4 weeks after inoculation or exposure. Interestingly, all the serum samples and all the nasal swabs were negative at his time. Interestingly, dividing the results of the RT-PCR analysis of the 10 sheep into two groups of 5 sheep (5 carriers and 5 non-carries) indicate, that the average number of days with swabs detected as positive were significantly higher in the carrier group for both nasal (increased with 3.5 days) and rectal swabs (increased with around 2 days) and furthermore, the average peak levels in these swabs were also higher for the carriers. The differences between these two groups in regard to their serum samples was less pronounced than for the swabs. Average viraemia was only slightly longer in the carriers (increased by less than 1 day), however the average peak viraemia was increased as for the swab samples (an average increase of about 4-fold). Thus, these studies indicate, that there is a direct correlation between peak viral load and duration of the viral load in serum and swabs on the subsequent development of carrier sheep.

The profile of infectiousness of FMD in sheep has been established based on a quantitative real time RT-PCR combined with virus titration of selected samples. Under our experimental conditions the curve of infectiousness was short and showing that when there is a high contact rate between sheep, transmission will occur rapidly, most likely by inhalation of aerosolized virus. The evidence that this does not always occur under field conditions is probably a reflection of the management conditions. Management activities which will increase the direct contact rate, and therefore transmission are for example housing for lambing or in connection with transport. Other conditions increasing direct contact are shearing and de-worming, the highest risk in this setting probably being the contamination of potential virus containing material onto damaged epithelium. Thus, transmission in sheep may have very different outcomes depending on the specific husbandry of a certain premises or a whole area. Thus, under intensive husbandry, sheep are likely to be kept at a high density and often the sheep spend at least part of their time inside. Thus, in such a system the possibilities for FMD transmission are maximal. However, in less intensive systems this may be very different. Stocking density is lower and most sheep will spend all/almost all time outside without solid housing. In such a system significant aerosol transmission is not very likely, because the potential concentration of FMDV in air never reach the minimal infective dosis (MID) level. Thus, it appears more likely, that in such cases, transmission will be much slower, and that the mechanism will involve contact transmission via infected excretions, as for instance vesicular fluid, nasal fluid or saliva/drool. However, it should be pointed out, that at the time of for example peak viral levels in nasal fluid the sheep, described in this experiment, had shown clinical signs of FMD, including lameness, and have had detectable vireamia for several days. In other words, while sheep to sheep aerosol transmission (short range) is difficult to control because airborne excretion occur before
clinical signs; it is most likely that transmission by virus-containing excretions later in the infectious process could in fact be minimized/controlled provided thorough examination of all sheep before and after movement.

In conclusion, available evidence suggests that conditions facilitating aerosol transmission, i.e., high animal density and closed housing with limited air-change, favor fast transmission in sheep, while, in contrast, conditions decreasing aerosol transmission, i.e., low density, out-door sheep herds with little contact among different groups of sheep, favor slow transmission among sheep.

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REFERENCES


**TABLE 1.** Doses of airborne virus excreted by the infected sheep.

<table>
<thead>
<tr>
<th>Group</th>
<th>Air sample</th>
<th>TCID/litre air</th>
<th>Excretion/amount present 24 hours TCID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>UI 97</td>
<td>May 1</td>
<td>0.7</td>
<td>4.74 for 2 sheep</td>
</tr>
<tr>
<td>UJ 03</td>
<td>Sample at 1 day pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UJ 01</td>
<td>May 2</td>
<td>0.27</td>
<td>4.34 for 2 sheep</td>
</tr>
<tr>
<td>UJ 00</td>
<td>Sample at 1 day pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UI 97</td>
<td>May 3</td>
<td>neg</td>
<td>ND</td>
</tr>
<tr>
<td>UJ 03</td>
<td>sample at 2 day pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UI 96</td>
<td>May 4</td>
<td>neg</td>
<td>ND</td>
</tr>
<tr>
<td>UI 99</td>
<td>sample at 2 day pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 10</td>
<td>Cyclone 1</td>
<td>neg</td>
<td>ND</td>
</tr>
<tr>
<td>sheep</td>
<td>Sample at day 1 pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 10</td>
<td>Cyclone 2</td>
<td>0.2</td>
<td>4.7 (likely from 4 contacts)</td>
</tr>
<tr>
<td>sheep</td>
<td>Sample at day 2 pi</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average aerosol excretion of $10^{4.3}$ TCID50/24 hour/sheep at 1 day after inoculation and below detectable levels on day 2. $10^{4.7}$ TCID50 for the box with 4 contact sheep (likely to excrete virus) equals $10^{4.1}$ TCID50 per (contact) sheep as measured in the box (reduced by air filtration) and thus to be equivalent to about $10^{4.5}$ TCID50 per 24 hour per contact sheep around day 2 (i.e. 1 day after the inoculated sheep).
TABLE 2. Doses of airborne virus excreted by the infected cattle.

<table>
<thead>
<tr>
<th>Collected in 20 min</th>
<th>in litre air</th>
<th>Estim. 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both cattle Cyclone 1 (PID 1):</td>
<td>252 TCID</td>
<td>3400</td>
</tr>
<tr>
<td>Both cattle Cyclone 2 (PID 1):</td>
<td>100</td>
<td>3400</td>
</tr>
<tr>
<td>Both cattle Cyclone 3 (PID 3):</td>
<td>252</td>
<td>3400</td>
</tr>
<tr>
<td>Both cattle Cyclone 2 (PID 3):</td>
<td>&lt;30</td>
<td>3400</td>
</tr>
</tbody>
</table>

Cyclone average per cattle is 4.24-4.35 logs per 24 hour per cattle, highest on day 1, i.e. 4.3 logs as for sheep, and slightly lower on day day 3, i.e. around 4.2 logs per 24 hours.