Review

Foot-and-mouth disease: A review of intranasal infection of cattle, sheep and pigs

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Abstract

In an outbreak of foot-and-mouth disease (FMD) it is important to identify animals at risk from airborne virus. Investigations have been carried out over the years to determine the dose required to infect cattle, sheep and pigs by the intranasal route. This paper reviews the results of investigations for animals which have been infected by instillation or spraying a virus suspension into the nostrils or by exposure to affected animals through a mask or by indirect contact.

The lowest doses were found by use of a mask. With virus from affected pigs given through a mask, doses of 18 infectious units (IU) in cattle and 8 IU in sheep were found to cause infection and give rise to lesions. Overall, cattle required the least amount of virus followed by sheep. Pigs required a dose of 22 IU to cause infection and a dose of 125 IU to give rise to lesions. In many experiments pigs failed to become infected. With all three species the dose varied with the individual animal and the virus strain. For modelling previous outbreaks and in real time, a dose of 8 IU or 10 and 50% infectious doses (ID50) could be used where cattle and sheep were involved. Experience in the field, combined with the results from experiments involving natural infection, indicate that pigs are not readily infected by the intranasal route. However, for modelling purposes a dose of about 25 IU should be used with care.

Investigations are needed to determine doses for virus strains currently in circulation around the world. In addition, the nature of the aerosol droplets needs to be analysed to determine how the respective amounts of infective and non-infective virus particles, host components and, in later emissions, the presence of antibody affect the survival in air and ability to infect the respiratory tract. Further work is also required to correlate laboratory and field findings through incorporation of the doses into modelling the virus concentration downwind in order that those responsible for controlling FMD are provided with the best available assessment of airborne spread. Finally, the doses found for infection by the intranasal route could be applied to other methods of spread where virus is inhaled to assess risk.

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Keywords: Foot-and-mouth disease; Intranasal infection; Review of experimental results

Introduction

Foot-and-mouth disease (FMD) is a highly infectious viral disease of cloven-hoofed animals both domestic and wild. The disease spreads by contact between infected and domestic animals, by animal products (milk, meat and semen), by mechanical transfer on people, wild animals and birds, by vehicles and fomites and by the airborne route. Spread by airborne carriage on the wind has been considered a possibility from the beginning of the 20th century especially by Scandinavians (Bang, 1912; Donaldson, 1979; Penberthy, 1901) and its part in spread of disease was recognised in the UK in the 1967–1968 and 2001 epidemics and in the spread of FMD from Brittany to the Isle of Wight in 1981 and in Hampshire in 1967 (Donaldson et al., 1982; Gloster et al., 2003, 2005a,b; Hugh-Jones and Wright, 1970; Mikkelsen et al., 2003; Sellers and Forman, 1973).
The airborne disease cycle can be divided into three stages: emission, transport and inhalation. Before 1984, the amounts of virus emitted by infected animals, the size of the infective particles and their survival in air was generally established but the process of inhalation, especially the dose required to initiate infection by the respiratory route, remained to be investigated more fully. A review of aspects of airborne spread of FMD is given in the paper on pathogenesis and diagnosis of FMD by Alexandersen et al. (2003b).

The potential for the spread of FMD by the airborne route can be determined by estimating the amount of virus released into the atmosphere and establishing the meteorological conditions in the vicinity of infected animals. This information is used as input into an atmospheric dispersion model which calculates downwind concentrations of virus and an inhaled dosage (Gloster et al., 2003; Ryall and Marson, 1998; Sørensen et al., 2000, 2001). To determine the area at risk from airborne infection the dosage to initiate infection is also required.

This paper provides a review of the published experimental data involving cattle, sheep and pigs with a view to giving guidance on dosage to those required to model the airborne risk of disease spread and to those responsible for controlling disease outbreaks. The dose determined can also be used to assess the risk of infection of the respiratory tract, where virus is inhaled from contact with affected animals, contaminated personnel, vehicles that have previously transported affected animals, aerosols from milk spils and from contaminated fomites.

Experimental conditions

The experiments can be divided into four categories:-

1. Application by instillation of virus suspension into the nostrils – artificial method of infection with artificially prepared virus.
3. (a) Exposure of animals to the aerosols of infected animals through a mask – artificial method of infection with naturally produced virus. (b) Exposure of cattle virus generated from a May spinning top through a mask – artificial method of infection with artificially prepared virus.
4. Indirect contact between infected animals and clean recipient animals – natural method of infection with naturally produced virus.

All of the experiments were carried out in isolation units under disease controlled conditions with animals from European breeds up to 3 years old. FMD strains of all virus types except SAT1 and SAT3 were used. The type of spray and the collecting apparatus varied between experiments.

FMD virus was titrated by different methods: intra-dermal inoculation of the tongues of cattle (Graves and Cunliffe, 1960; Henderson, 1952; Korn, 1957), unweaned mice (Eskildsen, 1969; Terpstra, 1972), sheep, lamb and pig kidney monolayer tissue cultures (Sutmoller et al., 1968; McVicar and Sutmoller, 1976, 1969; Bouma et al., 2004; Brown et al., 1992, 1996) and bovine thyroid monolayer (BTY) tissue cultures, which from 1981 onwards were used in the majority of experiments. BTY cultures had been found to be the most sensitive at detecting virus in air samples from affected animals (Donaldson et al., 1970). Strains adapted to pigs do not grow in BTY cultures and the pig kidney IBRS2 cell line was used instead (Dunn and Donaldson, 1997). In experiments from 2002 onwards, viral RNA in virus containing material was measured by the reverse-transcriptase polymerase chain reaction (RT-PCR) method.

The genome equivalents per millilitre were about 100–1000-fold higher than the titre of infective virus in BTY cells, although in late infection this increased up to 100-fold more, probably due to the presence of antibodies (Alexandersen et al., 2003a). This ratio between genome equivalents and infective virus is similar to the ratio of non-infective 25 nm particles and infective 25 nm particles (100–1000-fold to 1) found by electron microscopy (EM) of virus suspensions (Report, 1956-1960; Bradish et al., 1960).

Evidence of infection was taken as the presence of clinical signs, viraemia and seroconversion, or viraemia and seroconversion, or viraemia or seroconversion alone. Some FMD strains do not give rise to detectable lesions in cattle, pigs and especially in sheep (Alexandersen et al., 2003b). Viraemia can be of short duration without leading to the development of antibody (Garland, 1974; Gibbs et al., 1975; Donaldson and Kitching, 1989). Where animals were killed during the incubation period, the presence of virus in more tissues than earlier in the incubation period was taken as evidence of infection. Animals may also show transient levels of antibody (Alexandersen et al., 2002; Alexandersen and Donaldson, 2002). Animals with transient antibody levels 1 in 45 or greater were regarded as being infected. Animals without detectable lesions may pass on infection to others (Callens et al., 1998).

Review of animal experiments

The experimental results by all methods of infection for cattle, sheep and pigs are given in Tables 1–3 and summarised in Table 4 and Fig. 1. Experiments specifically designed to estimate dose are indicated in the tables (D). The remaining experiments were part of investigations on the pathogenesis of FMD, the exposure of vaccinated animals to infection and the development of carriers, in which the dose was also measured.
Table 1
Recipient animals – cattle

<table>
<thead>
<tr>
<th>Method</th>
<th>Virus type/strain</th>
<th>Virus source</th>
<th>Dose (logIU)</th>
<th>Amount</th>
<th>Experimental length</th>
<th>C</th>
<th>V</th>
<th>S</th>
<th>+ve</th>
<th>−ve</th>
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<td>2</td>
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<td>6</td>
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Spray

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<td>from 0.95, 1.25, 1.45, 2.25 BTY IU</td>
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<td>10 min</td>
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<td>4.25 BTY IU</td>
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<tr>
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<td>3 pigs</td>
<td>3.85 BTY IU</td>
<td>2 h</td>
<td>1</td>
<td>1</td>
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<td>3</td>
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<td>Indirect</td>
<td>O UKG2001</td>
<td>4 sheep</td>
<td>N/A</td>
<td>5 h</td>
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<td>1</td>
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<td>2</td>
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</table>

Key: C = clinical signs, V = viraemia, S = seroconversion or carrier, PK = pig kidney monolayer tissue cultures, SK = sheep kidney monolayer tissue cultures, LK = lamb kidney monolayer tissue cultures, BTY = bovine thyroid monolayer tissue cultures, IU = infectious units, p.i. = post infection, trans = transient, N/A = not available. The dose is the amount of virus inhaled at the nostrils, D = experiment to determine dose, ID50s are expressed as IU (ID50 × 0.7 assuming a Poisson distribution). The number in the penultimate column (no. Fig. 1) cross refers to the X-axis in Fig. 1.
Most experiments were carried out with type O strains, but type A, SAT 2 and Asia 1 strains were also used. In some successful experiments no end point was determined (Table 1). The lowest dose by instillation was found to be log2.0 IU (100 IU) (Sutmoller et al., 1968) and by spray was log1.95 IU (90 IU) (Henderson, 1952). In experiments using a mask, log1.25 IU (18 IU) from natural infection and log0.95 IU (9 IU) from spray from a May spinning
The lowest doses. The lowest dose to cause clinical lesions was log 1.25 IU (18 IU) after natural infection or spraying through a mask. The highest dose that failed to cause infection using a mask was log 1.55 IU (35 IU) for natural infection and log 1.25 IU (18 IU) from spray from a May spinning top (Donaldson et al., 1987). The lowest dose for indirect contact infection was log 2.25 IU (180 IU) (Donaldson and Stone, 1982) were the lowest doses. The lowest dose to cause clinical lesions was log 1.25 IU (18 IU) after natural infection or spraying through a mask. The highest dose that failed to cause infection using a mask was log 1.55 IU (35 IU) for natural infection and log 1.25 IU (18 IU) from spray from a May spinning top (Donaldson et al., 1987). The lowest dose for indirect contact infection was log 2.25 IU (180 IU) (Donaldson and Stone, 1982).
Kitching, 1989). Henderson (1952) found a difference between strains for the lowest dose to cause infection (Table 1), a finding that he correlated with differences in reaction to contact infection. In the experiments with masks the dose to cause infection varied with individual animals.

Sheep

Strains of types O, A and C were used in the experiments the majority being of type O. End points could not be demonstrated in every experiment, some showing successful infection, one other failure to infect by indirect contact (J.-F. Valarcher et al., unpublished data). The lowest dose for instillation was log 3.35 IU (2250 IU) (Hughes, 2002). In mask experiments the lowest dose was found to be log 0.9 IU (8.0 IU), which was also the dose to cause clinical infection. The highest dose that failed to cause infection was log 1.7 IU (50 IU) (Gibson and Donaldson, 1986). In experiments where sheep were exposed by indirect contact a dose of log 2.35 IU (225 IU) over 24 h was found to infect (Esteves et al., 2004). Differing values were found in the dose required to infect individual sheep after natural infection through a mask.

Pigs

The strains used in the experiment were of type O apart from C Noville. Instillation of log 6.25 IU (1800,000 IU) failed to give rise to lesions in pigs (Graves and Cunliffe, 1960). Virus was recovered from the tissues of pigs killed 72 and 96 h after receiving a dose of log 3.95 IU (9000 IU) (Terpstra, 1972). The lowest dose to cause infection in pigs exposed to natural virus through a mask was log 1.35 IU (22 IU), while the lowest dose to cause lesions was log 2.1 IU (125 IU) (Alexandersen et al., 2002). The highest dose to fail to cause infection after exposure to natural infection through a mask was log 3.35 IU (2250 IU) (Alexandersen and Donaldson, 2002). Experiments where pigs were exposed to natural infection with naturally pro-

Table 4

<table>
<thead>
<tr>
<th></th>
<th>Instillation</th>
<th>Spray</th>
<th>Indirect contact</th>
<th>Mask</th>
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<td>Cattle</td>
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<td>7100</td>
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<tr>
<td>Pigs</td>
<td>&gt;1,800,000</td>
<td>9000</td>
<td>&gt;250,000</td>
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</table>

1, minimum dose to initiate sub-clinical infection; 2, minimum dose to initiate clinical infection; 3, highest dose not to initiate infection.

Fig. 1. Summary of results from experiments which involved exposure of cattle, sheep and pigs to artificially prepared virus through a mask, natural virus through a mask or by indirect infection. The results from experiments involving cattle, sheep and pigs have been plotted separately. For each experiment the source and method of administering the virus is given (C = cattle, P = pig, S = sheep, * = spinning top, M = mask and I = indirect contact). Where in an experiment animals have been recorded as non-infected and infected the same symbol has been plotted (open = no infection, closed = infection). For example Gibson and Donaldson (1986) exposed sheep to virus from a pig (P), using a mask (M) and estimated the dosage for a number of animals some of which became infected (filled in squares) and others which remained uninfected (open squares).
duced virus by a natural method of infection – indirect contact – were not successful (Alexandersen and Donaldson, 2002; Aggarwal et al., 2002; Alexandersen et al., 2003a).

There was a difference between strain O UKG2001 and strain O SKR 1/2000 in the successful response and failure to respond respectively to infection through a mask by natural virus (Alexandersen and Donaldson, 2002). In the mask experiments there was variation in the responses of individual pigs to the doses given (Alexandersen et al., 2002; Alexandersen and Donaldson, 2002).

It can be seen in Fig. 1 that the lowest dose was found using a mask (artificial method, natural virus). There was little difference between cattle, sheep and pigs in the lowest dose to cause infection; however with pigs a greater dose than that for cattle was found to give rise to lesions. In addition a dose 60-fold greater than that for cattle failed to infect pigs (Alexandersen and Donaldson, 2002). With instillation, spray and indirect contact the doses found to infect cattle were considerably less than the doses required to infect pigs. Sheep also required a higher dose than cattle when infection was by instillation or indirect contact (Table 4).

Discussion

It may be thought that the absence of any standardized technique and the differences in housing, instrumentation and methods of measurement make it impossible to draw conclusions about the dose required by the intranasal route. However, in the majority of experiments reported from 1981 onwards, BTY tissue cultures (the most sensitive system) were used for virus assay. In addition, such experiments were reported from one centre (BBSRC, Pirbright), where from 1960 infected livestock were held in units under negative pressure, although the nature of the ventilation system changed over the years.

Instillation and spraying of virus preparations into the nostrils were the methods used to measure the dose in earlier experiments in cattle. In cattle the lowest doses by instillation (100 IU, Sutmoller et al., 1968), spray (90 IU, Henderson, 1952) and also by coughing and sneezing (100 ID50, Sellers et al., 1971) were similar. They were also lower than the doses found for pigs (Graves and Cunliffe, 1960 – instillation; Terpstra, 1972 – spray). In later experiments the development of the use of a mask ensured that virus from natural sources was introduced to the respiratory tract more effectively and the doses for cattle and pigs were found to be lower than those found by instillation and spray. As with instillation and spray in the earlier experiments the dose for cattle by administration through a mask was lower than that for pigs. In the field pigs are less likely to be infected through airborne spread than cattle. The doses used in previous modelling were based on those found in early experiments (Gloster et al., 1981; Donaldson et al., 1982). In later modelling, the lowest doses found in cattle and sheep by mask experiments (natural infection by artificial means) were used.

In investigations of doses of FMD virus strains in the future the use of a mask (artificial method of infection with naturally produced virus) would be the method of choice. The method most resembling that in the field (natural method with natural virus – indirect contact) did not result in lower doses and higher doses were found by artificial infection (instillation and spray). It should be pointed out that the majority of experiments by instillation, spray and indirect contact were not carried out to measure dose. The experimenters were using the method they found most appropriate to infect the animals.

The dose to initiate infection varied with the virus strain used and between individual animals. Some of this variation could be attributed to the fact that experiments were carried out under different conditions and with differing assay systems. Overall slight differences could be important in the field, where at low doses some highly susceptible individuals could become infected, whereas with other strains other individuals may escape infection resulting in the failure of disease spread.

Where exposure was by indirect contact (natural method and natural virus) factors in addition to animal species, individual animal and virus strain affected the success of infection by different doses. Such factors included the construction of the building and the airflow in the isolation unit. Successful infection by indirect contact was achieved in some of the experiments described here as well as by Fogedby et al. (1960), Burrows (1968), Garland (1974) and Sellers et al. (1968). Failure to cause infection in some of the experiments reviewed here was found by other investigators, such as Traub and Wittman (1957), where infection was directed from infected animals in a shed to calves and pigs. Bouma et al. (2004) found no evidence of infection when calves were exposed by indirect or direct contact to calves infected and reacting to the FMD strain of the 2001 Netherlands outbreak. However the lesser susceptibility of calves to the Netherlands virus as well as the environment could have been responsible for the failures.

Huber and Kitching (2000) analysed the spread of FMD within a cattle herd in Saudi Arabia and concluded that spread had occurred through aerosol between pens housing calves. This report also emphasized the importance of spatial or physical barriers in preventing cross-infection. Differences in inhalation of FMD virus by people in experiments in different laboratories could be explained by the presence or absence of air currents (Amass et al., 2003; Donaldson and Sellers, 2003). Air currents were found to assist the spread of viruses not known to cause airborne spread over distance, for example, African swine fever (ASF) and classical swine fever (CSF) (Dewulf et al., 2000; Wilkinson et al., 1977).

Successful infection in cattle and sheep occurred 1–10 min after doses of 8–18 IU given by mask. Sellers et al. (1970) found virus in the nose 5 min after contact with affected pigs. This indicated that if virus is present in the air it can be inhaled rapidly and give rise to successful infection in susceptible animals. Any virus particles that
fail to cause infection would be cleared from the respiratory tract and unlikely to build up until a critical dose is reached.

Infective aerosol particles inhaled through the nostrils pass through the nares and thence to sites in the respiratory tract, where the virus multiplies and spreads to other parts of the body (Burrows, 1972). The site of initial multiplication after inhalation of particles from natural sources has been found to be the pharyngeal area (Burrows et al., 1981; Alexandersen et al., 2003b). In the experiments described in this paper, particles from artificial sources initiated infection in the nasal mucosa and lung as well as in the pharyngeal area. In some papers the distribution of particles size between 2 and 10 μm is given, but owing to the range of sizes inhaled or given by spray it is not possible to determine from the results which size of particle initiated infection and in which part of the respiratory tract.

Gloster et al. (2006) decided that there was no need to take into account the size of the particles when modelling spread of disease in the field. However the size of aerosol particles and their site of initial infection in the respiratory tract are important in studying the pathogenesis of the disease. In addition, knowledge of particle size and likely site of infection are required for designing vaccines or antiviral substances for protection of the respiratory tract. The composition of individual aerosol particles may be important in determining the ability to initiate infection in the respiratory tract. It could be assumed by correlation with EM results that genome equivalents represent one infective virus particle to 100 or more non-infective particles. An aerosol particle may contain virus particles infective for BTY tissue cultures, non-infective particles, host material and, in the later stages of infection, antibody. Presence of antibody and high content of non-infective particles emitted from animals later in the infection may impair the ability to infect animals by the respiratory route. Further investigations of the nature of the aerosol particle are required.

Investigations have been made by modelling of past FMD outbreaks in UK in the Isle of Wight and in the 1967/68 and 2001 epidemics, where no cause other than airborne carriage of virus could be identified. The virus concentration at the site of the outbreak determined from the models was found to be up to 7000-fold less than the concentration that would lead to infection in cattle based on the dose of 10 TCID50 (Sorensen et al., 2000; Donaldson et al., 2001; Gloster et al., 2003, 2005a,b). The discrepancy between laboratory results and field findings could be due to lack of information on the output and timing of infection in animals and transmission including meteorological factors as well as to the dose inhaled. Finding that cattle and sheep can be infected by doses less than 18 IU and 8 IU, respectively, is unlikely to increase the concentration downwind significantly. Future research would be better directed at examining virus output and meteorological factors. The meteorological process, procedures and factors involved have been discussed elsewhere (Gloster et al., in press). See reference section for latest situation.

The minimal doses derived from the experiments can also be used to determine the risk through inhalation of aerosols derived from secretions and excretions from infected or contaminated animals, from contaminated people and contaminated vehicles, milk spills and fomites (Donaldson, 1979). The amounts of virus emitted in secretions and excretions are given in Sellers (1971), Thompson (1994) and Alexandersen et al. (2003b).

Conclusion

The experiments reported show that the lowest doses for cattle and sheep after natural infection were 18 IU and 8 IU, respectively. These values could be used for modelling airborne spread given the present state of knowledge. However investigations need to be carried out on the aerosol particle itself to define not only its components but also its capability both for infecting the respiratory tract at different stages of infection in the donor and for its survival over distance. The discrepancy between virus concentrations based on laboratory measurements of output and concentrations found downwind in field outbreaks indicates that further investigations on virus output and downwind transport are required. The experiments, together with field experience, indicate that spread within buildings may vary due to a number of factors. They raise questions about emission from and spread within buildings during an outbreak. The doses determined can also be used in determining the risk of aerosol spread in the field when methods of spread such as via contaminated vehicles and fomites are involved.

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Alexandersen, S., Quan, M., Murphy, C., Knight, J., Zhang, Z., 2003a. Studies of quantitative parameters of virus excretion and transmission

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