Influence of Exposure Intensity on Efficiency and Speed of FMD Transmission

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Abstract

FMDV can be spread by a variety of mechanisms including direct animal-to-animal contact, indirect contact facilitated by contaminated materials or airborne spread. The rate of spread, the incubation period until developing clinical signs of disease, as well as the severity of disease depends on many variables, including the dose received, the route of introduction, the virus strain, the animal species and the conditions under which the animals are kept. We previously presented data modelling infection in pigs directly inoculated with virus and also presented some experiments with varied intensity of contact among infected and susceptible pigs. Much more knowledge in regards to these variables is needed if model predictions are to be used in practical disease control. Here we extend our previous findings with more detailed studies in pigs exposed by contact to facilitate a better assessment of transmission risks.

Introduction

FMDV can be spread by a variety of mechanisms and the rate of spread and the incubation period as well as the severity of disease depends on many variables. These variables include the dose received, the route of introduction, the virus strain, the animal species and the conditions under which the animals are kept. We have previously presented data modelling infection in pigs directly inoculated with virus and also presented some experiments with varied intensity of contact among infected and susceptible pigs. Here we extend our previous findings with more detailed studies in pigs exposed by contact to facilitate a better assessment of transmission risks. Much more knowledge about these variables is needed if model predictions are to be used in practical disease control.

Materials and Methods

Animals and virus

One hundred and fifty four Landrace cross-bred Large White pigs weighing between 20 and 30 kg were used and inoculation was by heel pad injection or by contact as described previously (Alexandersen et al., 2001; Alexandersen et al., 2002a; Alexandersen et al., 2003b; Alexandersen & Donaldson, 2002). These pigs were part of experiments conducted for several purposes and the descriptions and results described in the present paper are directed towards describing transmission aspects. Twelve sheep were housed and inoculated in the coronary band or kept as contacts as described previously (Alexandersen et al., 2002b; Alexandersen et al., 2003b; Alexandersen & Donaldson, 2002). The usual dose for inoculation was approximately 0.25-0.5 ml of a 1:10 dilution of virus stock containing 10⁵.6-5.9 TCID₅₀ (BTY) of O UK 34/2001 virus (first pig passage). Contact pigs where kept in direct contact with inoculated (donor) pigs for various periods of time and in various numbers as described for the individual experiments. The progression of disease were assessed daily using a subjective scoring system (Alexandersen et al., 2003b; Quan et al., 2004). Body temperatures were recorded daily and blood and swab samples collected as needed. Animals
with severe lesions were killed by euthanasia. The experiments were performed sequentially and further details are described below.

**Pigs**

Experiment 1 (min-17). 20 pigs. In this experiment, 4 pigs were inoculated with \(10^{5.6}\) TCID50 of FMDV and used to infect four sets of 4 pigs by contact. The direct contact pigs were infected by being placed into the box with the 4 inoculated (donor) pigs for a period of 2 hours during which the pigs were allowed to mix freely as a group of 8. The contact pigs were then moved back to a clean box. The exposure was done on post inoculation (PI) days 1, 2, 3 and 4.

Experiment 2 (min-18). 24 pigs. As above, 4 pigs were inoculated with \(10^{5.6}\) TCID50 of FMDV but in this experiment used to expose contact pigs in a one-on-one setup (i.e. one inoculated and one contact pig together). Four boxes were each divided into 3 cubicles (a total of 12 cubicles) in which 1 contact pig/cubicle was placed. In each box, the sizes of the cubicles were \(1/4\) of the box for two of the cubicles and \(1/2\) for the third cubicle in each group. On day 1 PI, three inoculated pigs were placed in a box for a period of 2 hours with three contact pigs in a one-on-one setup. This process was repeated on day 2, 3 and 4 PI using the same inoculated pigs placed with unexposed contact pigs in different boxes. In addition, a further group of 4 direct contact pigs were mixed with the four inoculated pigs for 2 hours as a group rather than one-on-one on day 4 right after the one-on-one exposures.

Experiment 3 (min-19). 16 pigs. In experiment 3, two sets of 2 pigs were inoculated with \(10^{5.6}\) TCID50. In addition, 2 pigs were inoculated with \(10^{4.6}\) TCID50, and 2 pigs with \(10^{3.6}\) TCID50 of FMDV, respectively. Each set of two inoculated pigs were placed in a cubicle in an individual box (1/2 the area of a box). Two pigs were housed with each set of inoculated pigs as continuous contacts. Inoculated and contact pigs were allowed to mix freely and continuously during the experiment. Detailed results for the inoculated pigs have been described elsewhere (Quan et al., 2004).

Experiment 4 (min-20). 36 pigs. In this experiment, 18 pigs were inoculated with \(10^{5.6}\) TCID50. Another 18 pigs were housed with these as continuous contacts. The pigs were divided into 6 groups of 6 pigs in separate boxes, each group having \(1/2\) the area of a box and each group containing 3 inoculated pigs and 3 direct continuous contacts. Inoculated and direct continuous contact pigs were allowed to mix freely during the course of the experiment. One box initially contained 2 groups of 6 pigs, but as these pigs were killed within 2-6 hours for other purposes, we will only describe the results of the other 4 groups of 6 pigs. Two inoculated and two contact pigs in each group were killed on days 1-4 allowing a variable intensity of contact for the 4 groups.

Experiment 5 (min-21). 36 pigs. Experiment 5 was an identical repeat of experiment 4.

Experiment 6 (Pig DEFRA 2). Eight pigs were kept in \(3/4\) of a box (the other \(1/4\) box had 3 sheep, see below), and four of these pigs directly inoculated and the four others as direct contacts. At 2 days pi fourteen pigs in a separate box were exposed by moving the four inoculated pigs into their box for 2 hours.

**Sheep**

Experiment 1 (DEFRA-1). 3 sheep infected by inoculation in the coronary band.

Experiment 2 (DEFRA-2). This experiment was done concurrently with the DEFRA 2 pig experiment mentioned above, and for this sub-experiment, 3 sheep was placed in \(1/4\) of the box separated from the 4 inoculated and 4 contact pigs in the other part of the box by a metal gate which did not allow the animals to pass but freely allowed air to pass from one part of the box to the other. Direct contact was in principle possible through the metal gate, however, it appeared that the sheep and pigs did not try to have direct contact.

Experiment 3 (IE-JG-SA-Sheep-airb-1). The details of this experiment will be described in a separate presentation. Briefly, three sets of one recipient sheep were exposed to airborne virus from three sets of one inoculated donor sheep separated by wooden cubicles in 3 separate boxes.

**Assay for virus, genome and antibodies**

The infectivity in samples was assayed by inoculation of monolayer cultures of primary bovine thyroid (BTY) cells in roller tubes (Snowdon, 1966). The specificity of the cytopathic effect observed in cell cultures was confirmed by antigen ELISA (Ferris & Dawson, 1988; Hamblin et al., 1984; Roeder & Le Blanc Smith, 1987). Quantitative real-time 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 as described in detail elsewhere (Alexandersen et al., 2003b; Quan et al., 2004; Reid et al., 2003).
Serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to FMD virus (Ferris, 1987; Hamblin et al., 1986) and certain samples verified by virus neutralisation assay (as described in the Manual of Standards for Diagnostic Techniques and Vaccines, l’Office International des Epizooties, OIE, 2000).

Results

Pigs

Experiment 1 (min-17). This experiment was designed to test the efficiency and speed of spread of FMD in pigs exposed as a group of 4 inoculated (donor) and 4 contact pigs allowed to mix freely for a period of 2 hours. Exposure took place at PF days 1 to 4 for the individual groups. Inoculated pigs showed local lesions from 1 day pi and generalized lesions from 2 days pi and severe generalized disease from 3 days pi. For the group of contact pigs exposed at 1 day pi of the donor pigs, only 2 contact pigs showed any clinical signs of disease starting on days 7 and 9, respectively. These two pigs had a viraemia from days 4 and 6, respectively while the other two pigs in this group did not show any clinical signs nor a viraemia up to 11 days after exposure. For the other groups of contact pigs, all 4 contact pigs in each group showed clinical signs of disease starting on days 3, 5, 5 and 6, for the group of pigs exposed at 2 days pi; at days 4, 5, 5 and 9 for the pigs exposed at 3 days pi and on days 4, 5, 5 and 6 for the group of pigs exposed at 4 days pi. That the infectiousness of the donor pigs was very similar for the period from 2-4 days pi was further supported by the fact that the development of viraemia in these groups was virtually identical although perhaps with a slightly faster development in the contact pigs exposed at 3 days pi. This notion of slightly highest infectiousness of the day 3 pi donor pigs were further substantiated by testing of nasal swabs from either the donor or the contact pigs immediately after contact, which also showed the highest content of FMDV RNA on day 3 pi.

Experiment 2 (min-18). This experiment was essentially done as for experiment 1 with a 2 hour exposure on days 1, 2, 3 and 4 pi. The difference from experiment 1 was that pigs in experiment 2 were exposed one-on-one in cubicles with approximately the same space per pig as for the group exposure above. In addition, on day 4 pi a group of 4 contact pigs was also exposed to the donor pigs, i.e. in a group of 8 pigs. In this experiment the donor pigs showed generalized disease from 3 days and severe disease on day 4 pi. For the pigs exposed individually (one-on-one) none of the pigs exposed on days 1, 2 and 3 pi developed clinical signs of disease and none of them developed a viraemia although nasal swab results indicated some, perhaps abortive, local replication. For the pigs exposed one-on-one on 4 days pi, 2 out of 3 pigs showed clinical signs of disease starting on days 5 and 10, respectively and both had a viraemia starting on days 1 and 7, respectively. The third pig in this group did not develop clinical disease nor a viraemia. Although only a single observation, this pig was exposed in the “large cubicle”, i.e. 1/2 box while the other two were exposed in “small cubicles”, i.e. 1/4 box. Of the pigs exposed as a group on day 4 pi (4 donors plus 4 contacts) 3 out of 4 pigs developed clinical signs of disease starting on days 4, 5 and 5 and viraemia from days 1,2 and 3, respectively. The 4th pig in this group did not show any clinical signs but clearly had a subclinical infection manifested by a low grade viraemia lasting from day 5 to 11 post exposure. That the infectious dose for the pigs exposed individually was higher than for the pigs exposed individually was further supported by the fact that the development of viraemia and FMDV RNA load in nasal swab samples in the pigs exposed as a group was faster than in the pigs exposed individually. Based on the load of FMDV RNA in nasal swabs from the donor pigs and the contact pigs, the pigs exposed at 1 and 2 days pi received a very low dose while the pigs exposed at 3 and 4 days pi received a high dose (the dose the day 4 pi contact pigs received was slightly higher than the dose the day 3 pi contact pigs received). Interestingly, the pigs exposed individually on day 4 received an almost identical FMDV RNA load in the nasal swabs as the pigs exposed as a group, however, while the individually housed pigs appeared to initially almost clear their virus, the pigs housed as a group where more likely to show a rapid increase in virus load.

Experiment 3 (min-19). Detailed results for the inoculated pigs have been described elsewhere (Quan et al., 2004). Out of the two times two pigs inoculated with 10^{5.6} TCID50 of FMDV one showed lesions from day 1 pi and the other 3 pigs from day 2 pi with severe disease in all 4 pigs from day 2-3 pi. The 2 pigs inoculated with 10^{6.6} TCID50 showed severe clinical disease from day 2 pi, essentially as seen for the higher dose pigs. Of 2 pigs inoculated with 10^{5.6} TCID50, one had lesions from day 1 and the other from day 3. The impression from this experiment was that with intradermal (heel pad) inoculation within this dose interval the time to clinical disease or to viraemia did not differ significantly, however, at the low dose the outcome was less predictable with the possibility of a slower development of disease in some inoculated pigs, i.e. one pig showed a delayed development of viraemia (starting at 2 days as opposed to 1 day in the other pigs) and clinical disease (starting at 3 days as opposed to 1-2 days in the other pigs). Development of disease was very similar in the 3 groups of contact pigs, with clinical disease starting on days 3-4 and viraemia starting on days 1-2. Also, levels of FMDV in nasal swabs were very similar. It is of interest to note, that the disease
development in the two contact pigs exposed to the 2 donor pigs inoculated with the low dose were indistinguishable from the other groups.

Experiment 4 (min-20). The results described here cover the results for the 4 groups of contacts as the results for the inoculated pigs were similar for the 4 groups with viraemia starting at day 1 and clinical disease starting at days 1-2 pi (Quan et al., 2004). The four groups of 6 pigs (3 inoculated and 3 contact pigs together) were housed in 4 individual boxes. Two of the inoculated pigs in each box were removed on day 1, 2, 3 and 4, respectively while the third inoculated pig in each box was kept in the box. In the box where all 3 donor pigs were kept until day 4 pi, all 3 contact pigs developed viraemia starting at day 2 and clinical disease starting on day 3 and becoming severe from day 4-5. In the box where 2 donor pigs were removed at 3 days pi, two pigs had a viraemia but not yet clinical disease on day 3 when they were killed, while the last contact pig in this box did not develop any clinical signs of disease when monitored up to 22 days after exposure. In the box where 2 donor pigs were removed at 2 days pi, two pigs had a viraemia but not yet clinical disease on day 2 when they were killed, while the last contact pig in this box developed clinical disease on day 5 pi. In the last group in this experiment, two of the donor pigs were removed at 1 day pi and none of 2 contact pigs killed at 1 day pi had viraemia, FMDV RNA in nasal swabs or clinical disease while the last contact pig in this group developed a viraemia and clinical disease from 5 days pi. Examination of nasal swabs showed that viral RNA loads were virtually identical in donor and contact pigs when kept under these conditions.

Experiment 5 (min-21). This experiment was a repeat of experiment 4 described above and involved four groups of 6 pigs. In this experiment the development of viraemia and clinical disease in the inoculated pigs were particularly fast and thus slightly faster than in experiment 4, above. In the box where all 3 donor pigs were kept until day 4 pi (NB, 1 donor pig killed at day 2 pi due to very severe clinical disease), all 3 contact pigs developed viraemia starting at day 2 and clinical disease starting and becoming severe on days 3-4. Similarly, in the box where 2 donor pigs were removed at 3 days pi, the 3 contact pigs had a viraemia and severe clinical disease on day 3. In the box where 2 donor pigs were removed at 2 days pi, two pigs had a viraemia but not yet clinical disease on day 2 when they were killed, while the last contact pig in this box developed clinical disease on day 3 pi. In the last group in this experiment, two of the donor pigs were removed at 1 day pi and none of 2 contact pigs killed at 1 day pi had viraemia or clinical disease but did have FMDV RNA in nasal swabs. The last contact pig in this group developed clinical disease from 3 days pi.

Experiment 6 (Pig DEFRA 2). The donor pigs developed viraemia and clinical disease within 1-2 days pi while the continuous contact pigs developed viraemia from 2 days and clinical disease from 3 days after inoculation of the donor pigs. The pigs kept in contact for 2 hours at 2 days pi developed a viraemia at 1-2 days and severe clinical disease at 2-3 days after exposure.

**Sheep**

Experiment 1 (DEFRA-1). One inoculated sheep developed clinical disease on day 1, another sheep on day 2 and the last inoculated sheep clinical disease on 3 days pi. All 3 sheep had increased body temperature from day 2-4 pi.

Experiment 2 (DEFRA-2). In this experiment the sheep started to develop a viraemia from 2-3 days after inoculation of the pigs and had peak viraemia and increased body temperature at day 4 and clinical disease from day 6.

Experiment 3 (IE-JG-SA-Sheep-airb-1). The inoculated donor sheep showed fever and typical clinical signs in the form of minor vesicular lesions along the coronary band, the interdigital space and the heel area within 1-2 days after inoculation. The recipient sheep were exposed to an estimated dose of airborne FMDV of around 300 TCID50 over a 24 hour period and two out of the three recipient sheep developed antibodies against FMDV at days 14-35 p.i.. The same two sheep were also positive for virus by testing of probang samples on days 23, 25, 28 and 35 p.i.. The recipient sheep did not show any clinical signs (only subclinical infection) and only one recipient sheep had a fever 4 to 7 days after inoculation of the donor sheep.

**Discussion**

Experience from the field and from experimental infections indicates that the speed and efficiency of FMD transmission is highly variable depending on biological characteristics of the specific FMDV strain involved as well as the prevailing husbandry conditions (Alexandersen et al., 2001; Alexandersen et al., 2002b; Alexandersen et al., 2003a; Alexandersen et al., 2003b; Bouma et al., 2004; Hughes et al., 2002b; Hughes et al., 2002a; Quan et al., 2004). Consequently, apart from the intrinsic biological virus attributes as well as the stochastic uncertainty inherent in the early phases of an outbreak, the...
development of an outbreak into a potential epidemic and the course of this epidemic, is extremely complex and difficult to accurately predict unless local factors are taken into account.

Pig experiment 1 described here clearly showed that when exposed as a group (4+4) the inoculated donor pigs were considerably less infectious at day 1 pi as compared to days 2-4 pi. On day 1 pi only two out of 4 contact pigs developed viraemia and clinical signs at day 7 and 9, respectively while the two other contact pigs did not show a viraemia nor clinical signs. In contrast, on days 2-4 pi all the contact pigs developed clinical disease starting around 5 days after exposure. Although no clear difference in development of the infection in the contact pigs exposed to inoculated pigs at days 2-4 pi was observed, a slightly higher infectiousness of the day 3 pi donor pigs was indicated by a slightly faster development of viraemia and a higher viral load in nasal swabs immediately after exposure of the contacts.

The results of the group exposure described above should be comparable with the one-on-one experiments described in pig experiment 2 although the development of infection and clinical disease in the donor pigs was slightly slower in experiment 2. Interestingly, in this one-on-one setup, transmission only occurred at 4 days pi but not on days 1-3 pi. Also, at 4 days pi the infection was only transmitted to 2 out of 3 contact pigs with clinical disease on days 5 and 10 after exposure, respectively and with no viraemia or signs of clinical disease in the third contact pig (followed for 14 days). In the pigs exposed as a group (4+4) at 4 days pi in this experiment, 3 out of 4 pigs developed viraemia from days 1-3 and clinical disease from days 4-5 while the fourth pig developed a subclinical infection with no clinical signs of disease but with a low level viraemia from days 5-11 after exposure. Interestingly, at 4 days pi the pigs exposed individually received an almost identical FMDV RNA load in the nasal swabs as the pigs exposed as a group, however, while the individually housed pigs appeared to partly clear their virus initially, the pigs housed as a group where more likely to show a rapid increase in virus load and to develop rapid disease reinforcing the influence of stocking density on both transmission efficiency but also on the development of clinical disease as previously hypothesized (Quan et al., 2004). Interestingly, although the pigs exposed at days 1-3 pi did not develop clinical disease nor viraemia, they appeared to have some, perhaps abortive, viral replication as suggested by viral RNA in nasal swabs. Consequently, these two experiments clearly show the difference in infectiousness over time and also the difference between pigs being exposed as a group or as one-on-one. Pig experiment 3 looked at whether the dose for inoculation of the donor pigs had importance for the subsequent development of disease in the contacts. However, the result indicated that the inoculation dose is mainly of importance in determining the consistency of infection in the donor pigs, low doses being less consistent, but that transmission of infection is still highly efficient provided that the donor pigs develop fulminant infection and that the contacts are exposed as a group.

Pig experiment 4 and 5 explored whether minor differences in combined exposure level and intensity could be measured in the current experimental set-up. The results of experiment 4 were not clear but did indicate that lowering the density in a group of pigs during the early stages of infection may slightly prolong the incubation period (from around 3 days to around 5 days) and may result in the development of subclinical infection in some exposed pigs. However, this effect could not be reproduced in experiment 5, most likely due to the very rapid development of infection in the donor pigs in this experiment resulting in efficient spread of disease to the contact animals before reduced pig density was achieved.

Pig experiment 6 showed like in previous experiments, i.e. pig experiment 1 reported here and other experiments with a number of FMDV isolates reported previously (Alexandersen et al., 2001; Alexandersen et al., 2003b; Alexandersen & Donaldson, 2002) that transmission and development of FMD is very rapid and efficient when pigs are exposed as a group, even when only exposed for a short time provided the excretion levels of the donor pigs are high.

The sheep experiments essentially showed that development of infection and clinical disease may be variable. The experiments described previously indicated that the incubation period in sheep exposed by close direct contact at high density and indoors may be as short as 1-2 days (Alexandersen et al., 2002b) while we here show that exposure by indirect contact to pigs result in an incubation period of around 6 days while exposure to a low dose of airborne FMDV (~300 TCID50 over a 24 hour period) resulted in no clinical signs of disease except for a slight increase in temperature at 4-7 days after exposure in 1 out of 3 exposed sheep although 2 of the 3 sheep became subclinically infected and carried the virus in probang fluid samples for at least 35 days after exposure.

Our studies together with previously published results and findings in the field confirm our previous notion that a strong correlation exists between dose (i.e. infectiousness of source and intensity of contact) and length of incubation period, severity of clinical disease and efficiency of spread. Furthermore, low intensity transmission may increase the risk of subclinical or abortive infection
which easily may escape clinical examination. Clearly, local conditions are of immense importance in
determining efficiency and speed of FMD transmission and can be an important determinant for the
outcome of an initial outbreak and a considerable source of inaccuracy if not truthfully accounted for
in mathematical models of epidemiological spread. In effect, such model based disease control can at
present only meaningfully be attempted by including detailed veterinary expertise and local

Conclusions

• The number of animals kept together in direct contact and the timing and length of contact
influence the incubation period, the efficiency of spread and the risk of subclinical or abortive
infection.

Recommendations

• More studies should be done using varying conditions and different strains of virus to provide
a better understanding of the epidemiology of FMD. Such data are urgently required for
development of realistic simulation models of disease spread.

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