Chapter 6

Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows

K. Orsel*[1], A. Bouma[1], A. Dekker[2], J.A. Stegeman[1] and M.C.M. de Jong[3]

1. **Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands**
2. **Central Institute for Animal Disease Control Lelystad (CIDC-Lelystad), Wageningen UR, The Netherlands**
3. **Quantitative Veterinary Epidemiology, Wageningen University and Research Centre Wageningen, The Netherlands**

Abstract
Detection of clinical signs of foot and mouth disease (FMD) is important for rapid detection of new outbreaks. However, virus excretion could start before clinical signs become apparent and the infection could also be sub-clinically. Therefore, we quantified experimentally observed transmission that occurred before the first clinical signs were noticed, by the reproduction ratio $R_{incubation}$. $R_{incubation}$ is defined as the average number of secondary infections caused by one infectious individual before clinical signs become apparent. It is assumed that individual hosts, before or without showing clinical signs, are less infectious due to lower virus excretion. We therefore estimated the transmission of FMD proportionally to the virus excretion.
For both vaccinated and non-vaccinated groups of lambs and calves estimates for $R_{incubation}$ were below 1, indicating that only few secondary infections occurred, before clinical detection of the initially infected individuals. Estimate for $R_{incubation}$ for non-vaccinated dairy cows was $R_{incubation}$ =1.72 (0.41;7.13). In both non-vaccinated and vaccinated piglets estimates were above 1 ($R_{incubation}$ =13.20 and $R_{incubation}$ =1.26, respectively). These findings suggested that only in non-vaccinated pig herds possibly a large number of animals might have been infected before clinical signs are noticed.

1. Introduction

Foot and mouth disease (FMD) is a notifiable disease of cloven-hoofed animals, because it is highly contagious and can cause severe economic losses in livestock industry. Since a FMD virus introduction can never be fully prevented, monitoring for early detection of an infection is necessary to minimise the number of secondary outbreaks. Monitoring is mainly based on detection of clinically suspected cases. However, it is well known that infected animals can shed virus during the incubation period (i.e. time between infection and first detectable clinical signs), and in some species a FMDV infection can occur sub-clinically (i.e. infection without clinical signs) [1].

The role of infected but not yet detected individuals and herds has shown to be important, like during the UK epidemic in 2001, where epidemiological investigations suggested that by movement of undetected infected sheep before the first case was confirmed, up to 79 premises were likely exposed to a FMD infection [2]. In sheep, the incubation period is often longer compared to cows and pigs, and also the total duration of virus excretion in sheep can exceed virus excretion in cattle and pigs. The highest virus titers on the other hand are excreted by pigs followed by cattle and sheep. In scenario studies, the number of secondary outbreaks in the high-risk period (i.e. the time between introduction of FMD virus into a country and detection) is often based on the reproduction ratio estimated for the whole infectious period of an infected animal. It is unknown however, how many secondary infections are to be expected in the incubation period. As virus excretion patterns differ between various species, as well as the appearance and severity of clinical signs, the question is whether these virus excretion patterns do result in differences in transmission before clinical signs are observed.

The aim of this study was therefore to estimate transmission of FMDV among vaccinated and non-vaccinated calves, lambs, piglets and dairy cows in the incubation period. We calculated the transmission parameter, proportional to the virus excretion, both during incubation period ($R_{incubation}$) and the full experimental period ($R_{fullperiod}$).

2. Methods

2.1 Experimental data

The data used originated from transmission experiments, as was previously described in more detail [5-8]. In total 52 calves, 52 lambs and 40 piglets, all 10 weeks of age, and 40 multiparous lactating dairy cows were included. In the transmission experiments the animals were housed in groups, in which half of the

group was inoculated with FMDV (approximately $10^{4.6}$ plaque forming units (pfu) of FMDV field isolate O/NET/2001). The other half of the group was contact exposed to the inoculated animals. All experiments were carried out with groups of non-vaccinated animals and groups of animals that were vaccinated once with (DOE) O_1Manisa vaccine, 14 days prior to challenge. Each experimental group of animals could mingle freely in its pen. Virus excretion was observed by daily collection of oro-pharyngeal fluid (OPF) samples. FMDV titers (pfu/ml OPF) were determined using plaque count on a monolayer of secondary lamb kidney cells [9].

2.2 Clinical signs

In each experiment an individual daily registration of all clinical signs was performed. For determination of clinical onset of a FMDV infection, observations of vesicles in the epithelium of the mouth, the tongue, teats, interdigital spaces and the coronary bands were taken into account.

2.3 Statistical methods for quantification of transmission in the incubation period ($R_{incubation}$)

The reproduction ratio $R_{incubation}$ was defined as the average number of new infections per infectious individual in the incubation period. We also included sub-clinically infected animals, in which virus excretion in OPF was observed. $R_{incubation}$ was estimated for each species both in vaccinated and non-vaccinated groups with transmission data proportional to virus excretion of individual animals in OPF. We assumed equal individual amounts of OPF produced by one species and that each pfu was equally infectious, both before and after clinical signs were observed.

At first a transmission rate β was calculated, which here represents the average number of newly infected animals per day per unit (pfu) of virus excretion in OPF. For this calculation we determined the mean daily virus excretion per pen (MDVE) since transmission was also determined per pen. The estimated β was based on the full experimental period.

The stochastic S-I-R model (susceptible-infectious-recovered model) as described by De Jong and Kimman [10] was adapted to a model based on the number of newly infected (cases), contact exposed animals (susceptibles) and the pen-wise summed daily virus excretion (for the "I-category"). We used a generalised linear model analysis with 'cases' as dependent variable, 'susceptibles' as binomial total, log (MDVE * Δt /N) as offset (where N is total number of animals in the experimental unit), and a binomial distribution. By dividing through N, we assume mass action, and transmission to be proportional to the density of infected and susceptible animals [11].

By multiplying the average virus titer per infectious animal with the average duration ($\overline{T}$ in days) of virus excretion either before clinical signs (for the $R_{incubation}$) or for the full period (for $R_{fullperiod}$) with this β, transmission was quantified:

$$R(incubation) = \beta * \frac{virustiter}{infectious\,animal} * \overline{T}$$

The contribution of an individual animal with clinical signs before virus excretion or without any virus excretion, to duration $\overline{T}$ was considered 0, since that individual did not contribute to the total virus excretion before clinical signs appeared. When no clinical signs were observed, the total duration of virus excretion of that individual was used. In addition to $R_{incubation}$ we also calculated a reproduction ratio for the whole experimental period, $R_{fullperiod}$ which was calculated with the pen-average of the total duration (in days) of virus excretion for T, independent of clinical observations. The duration of infectivity was calculated from the first till the last day that virus was present in OPF. Days that tested negative within this period were considered positive.

3. Results

3.1 Virus excretion and clinical signs

In figure 1, duration of virus excretion before clinical signs become apparent is illustrated. Note that the zero-category includes individuals where virus excretion started after clinical signs became apparent. In dairy cows and in non-vaccinated and vaccinated pigs a rapid start of virus excretion after inoculation and short incubation period were observed. In non-vaccinated and vaccinated lambs and calves the onset and duration of virus excretion was observed over a longer period of time. In the non-vaccinated groups of animals 16 out of 24 calves, 12 out of 24 lambs and all dairy cows and piglets showed clinical signs. In the vaccinated groups 1 out of 24 calves and 10 out of 20 vaccinated piglets showed clinical signs, whereas no clinical infection was observed in the lambs or dairy cows. A summary is given in table 1, to indicate whether virus excretion started either before or after clinical appearance, or when the infection was sub-clinical. Three non-vaccinated dairy cows, one vaccinated and six non-vaccinated piglets were euthanized for welfare reasons, due to severe FMD infection.

Figure 1: Duration of virus excretion in the incubation period per species

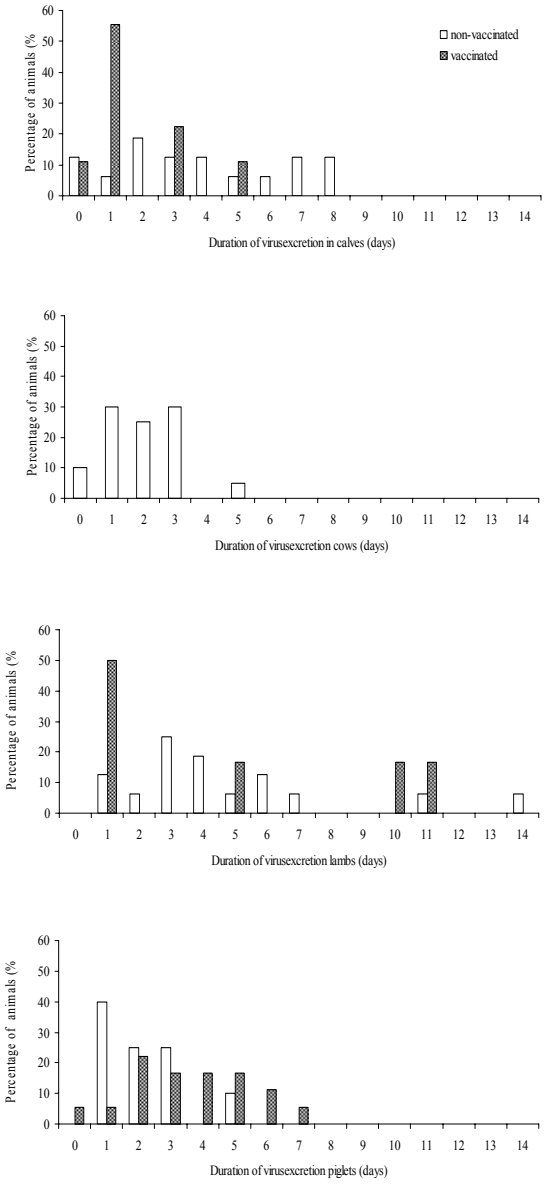

Table 1: Virus excretion related to observation of clinical signs (number of animals per category)

	Calves (nv)	Calves (vac)	Dairy cows (nv)	Lambs (nv)	Lambs (vac)	Piglets (nv)	Piglets (vac)
Virus excretion started before clinical appearance	11	0	18	12	0	20	9
Virus excretion started after clinical appearance	2	1	2	0	0	0	1
Virus excretion, no clinical signs	3	8	0	4	6	0	8
No virus excretion, clinical appearance	3	0	0	0	0	0	0
No virus excretion, no clinical signs	5	15	0	8	18	0	2

vac = vaccinated
nv = non-vaccinated

3.2 Reproduction ratio $R_{incubation}$

The virus transmission before clinical manifestation ($R_{incubation}$) was estimated for the non-vaccinated and vaccinated groups of calves to be 0.30 [0.03;3.43] and 1.03 10^{-8} [0;∞] respectively. For the non-vaccinated cows $R_{incubation}$ was 1.72 [0.41;7.13]. In the vaccinated dairy cows no virus excretion was detected so $R_{incubation}$ could not be calculated. In the groups of lambs $R_{incubation}$ was 0.21 [0.02;2.48] in the non-vaccinated and 0.16 [0.009;2.96] in the vaccinated groups, whereas for pigs the estimation for the non-vaccinated groups was 13.20 [4.08;42.68] and 1.26 [0.18;8.96] for the vaccinated groups. These results, including estimations for $R_{fullperiod}$ are summarized in table 2.

Table 2: Virus transmission as quantified with reproduction ratio R

Species	Vaccination status	R [a] 95% confidence limits	$R_{fullperiod}$ 95% confidence limits	$R_{incubation}$ 95% confidence limits
Calf	Non-vaccinated	2.52 [1.13;52.1]	0.67 [0.05;9.98]	0.30 [0.03;3.43]
	Vaccinated	0.18 [0.01;1.2]	$1.07\ 10^{-8}$ [0;∞]	$1.03\ 10^{-8}$ [0;∞]
Dairy	Non-vaccinated	∞ [1.3; ∞]	5.87 [2.67;12.89]	1.72 [0.41;7.13]
cow	Vaccinated	0 [0;3.4]	- [b]	- [b]
Lamb	Non-vaccinated	1.14 [0.3;3.3]	0.60 [0.03;13.10]	0.21 [0.02;2.48]
	Vaccinated	0.22 [0.01;1.78]	0.16 [0.009;2.96]	0.16 [0.009;2.96]
Piglet	Non-vaccinated	∞ [1.3; ∞]	30.74 [11.09;85.17]	13.20 [4.08;42.68]
	Vaccinated	2.42 [0.9;6.9]	1.44 [0.22;9.31]	1.26 [0.18;8.96]

R = Reproduction ratio; average number of secondary cases arising from one infectious individual estimated with the final size of infection

$R_{fullperiod}$= average number of secondary cases based on the virus excretion in OPF for the whole infectious period

$R_{incubation}$ = average number of secondary cases proportionally to the virus excretion in OPF before clinical signs become apparent

[a] Reproduction ratio calculations are described in more details in previous publications by Orsel et al.

[b] R cannot be calculated as the vaccinated dairy cows did not excrete virus in oro-pharyngeal fluid

4. Discussion

Clinical signs are important in the rapid detection of new outbreaks of FMD. However, infected animals can excrete virus before clinical signs become apparent, or sometimes a FMD infection occurs sub-clinical. Since secondary outbreaks may occur within the incubation period, we estimated the transmission of FMDV infections before the start of clinical signs by $R_{incubation}$. All transmission experiments were performed with FMDV strain O/NET/2001, which allows species comparison, but is a limitation for the extrapolation to different strains, since FMDV strains are known to be very antigenic varied, with different species specificity [12, 13].

For both non-vaccinated and vaccinated calves and lambs $R_{incubation}$ was estimated below 1. Within these types of herds, on average less than one new infection occurred, before clinical detection of the initially infected individual. Consequently, although clinical signs are often more difficult to be recognized in

calves and lambs [14], clinical monitoring seems suitable to detect infected herds at a time when the number of infected animals within the herd is still small.
Since the effect of virus excretion pattern (both height and duration of virus excretion) [15, 16] on virus transmission was never quantified before, discussions about the risk of virus transmission without clinical signs, especially in sheep, led to confusing conclusions. On one hand, it was concluded that sheep play a rather limited role in virus transmission because low virus titer are excreted and the disease often occurs sub-clinical [17]. On the other hand it was stated that because sheep show limited clinical signs, it makes them difficult to recognize, and consequently sheep may pose a major risk for virus transmission to occur [14]. Our estimations for $R_{incubation}$ take into account the differences in time till clinical signs were observed, and also the calculations were proportional to the amount of virus excreted in OPF. These estimations support the suggestion that sheep may play a limited role in virus transmission. We therefore conclude that infected sheep herds will probably be detected in time when monitored clinically.

Given high estimates of $R_{incubation}$ for FMDV infections in non-vaccinated pigs and estimates above 1 for dairy cows and vaccinated piglets, by the time clinical signs became apparent, transmission will most likely have occurred to other animals within the herd. This estimate was not based on the final size of infection, but used the transmission rate β, virus excretion and duration of virus excretion. When reproduction ratio estimates are based on the final size of infection (R_v and R_{nv} for vaccinated and non-vaccinated animals, respectively) other observations like serological responses are also taken into account. Consequently, those R_v and R_{nv} estimates will generally be higher than the $R_{fullperiod}$ reported here and they are most likely an overestimation of the true R value [18]. The transmission parameters $R_{incubation}$ and $R_{fullperiod}$ were based only on virus excretion in OPF and might underestimate the transmission per virus excreted, since virus excretion in milk is not taken in to account, which is a well known route of virus transmission [19].
We assumed equal OPF production between animals per day, but clinically infected animals often excrete more saliva, which may increase exposure of penmates to this infectious excretum. This may especially counts for the clinical period, although we assumed equal infectivity per amount of virus excreted throughout the experimental period. Most likely infectivity is lower in the incubation period compared to the clinical phase, which might have led to an overestimation of $R_{incubation}$.

In case $R_{incubation}$ is low, resulting in limited, if any, transmission in the herd before the animal is detected, it might be expected that the probability of FMDV transmission to another herd by that time is even lower, since the contact structure

between herds is less direct and intense compared to the within herd contact structures. For extrapolation it needs to be considered that estimates for $R_{incubation}$ are based on experimental infections, in which clinical disease is observed at animal level and might be recognized easier compared to field infections. In field situations however, observations are at herd level in which more infected animals can be present which increases the chance of clinical recognition of an infected herd. Important however, is a fast detection and report of suspicious clinical signs observed by the farmer.
Our estimates for transmission in dairy cows and piglets within herds are above 1. Especially in non-vaccinated herds with one clinically affected piglet most likely more infected individuals will be present, which makes the herd infectious to other herds. When culling capacity is limited, priority should be given to non-vaccinated herds of piglets, non-vaccinated herds of dairy cows followed by vaccinated piglets and other infected herds of susceptible species.

In conclusion, within herd transmission is most likely to occur in non-vaccinated piglets before clinical signs are observed. In contrast, at the moment of clinical detection of an infected herd with non-vaccinated dairy cows and vaccinated pigs only a limited number of secondary infections are likely to have occurred. Most likely clinical inspection in herds with lambs or calves is in time to prevent within herd transmission.
Finally, our results also give useful estimates of transmission before and after clinical detection which can be used for the modelling of detection and culling strategies in combating outbreaks of FMD [20-22].

Acknowledgements
This work was funded by the European Union SSPE-CT-2003-503603 and the Ministry of Agriculture, Nature and Food quality in The Netherlands.

References

1 Blanco E, Romero LJ, El Harrach M, Sanchez-Vizcaino JM. Serological evidence of FMD subclinical infection in sheep population during the 1999 epidemic in Morocco. Vet Microbiol 2002;85(1):13-21.
2 Mansley LM, Dunlop PJ, Whiteside SM, Smith RG. Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. Vet Rec 2003;153(2):43-50.
3 Kitching RP. Clinical variation in foot and mouth disease: cattle. Rev Sci Tech 2002;21(3):499-504.
4 Kitching RP, Hughes GJ. Clinical variation in foot and mouth disease: sheep and goats. Rev Sci Tech 2002;21(3):505-12.

5 Orsel K, Dekker A, Bouma A, Stegeman JA, Jong MC. Vaccination against foot and mouth disease reduces virus transmission in groups of calves. Vaccine 2005;23(41):4887-94.
6 Orsel K, Dekker A, Bouma A, Stegeman J, de Jong M. Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination. Vaccine: 2006; Accepted for publication.
7 Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. The effect of vaccination on foot and mouth disease virus transmission among dairy cows. Vaccine 2007;25:327-35.
8 Orsel K, de Jong M, Bouma A, Stegeman J, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after contact exposure to virus shedding pigs. Submitted for publication.
9 Bouma A, Dekker A, de Jong MC. No foot-and-mouth disease virus transmission between individually housed calves. Vet Microbiol 2004;98(1):29-36.
10 De Jong MC, Kimman TG. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 1994;12(8):761-6.
11 Bouma A. Transmission of pseudorabies virus within pig populations is independent of the size of the population. Preventive veterinary medicine 1995;23:163-77.
12 Doel TR. Natural and vaccine-induced immunity to foot and mouth disease: the prospects for improved vaccines. Rev Sci Tech 1996;15(3):883-911.
13 Dunn CS, Donaldson AI. Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. Vet Rec 1997;141(7):174-5.
14 Barnett PV, Cox SJ. The role of small ruminants in the epidemiology and transmission of foot-and-mouth disease. Vet J 1999;158(1):6-13.
15 Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of Quantitative Parameters of Virus Excretion and Transmission in Pigs and Cattle Experimentally Infected with Foot-and-Mouth Disease Virus. J Comp Pathol 2003;129(4):268-82.
16 Alexandersen S, Zhang Z, Reid SM, Hutchings GH, Donaldson AI. Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001. J Gen Virol 2002;83(Pt 8):1915-23.
17 Donaldson A. The role of sheep in the epidemiology of foot-and-mouth disease and proposals for control and eradication in animal populations with a high density of sheep. In: European commission for the control of Foot and Mouth disease of The food and agriculture organization of the united nations; 2000 5-8 September 2000; Borovets, Bulgaria; 2000. p. 107-16.
18 Velthuis A, Bouma A, Katsma W, Nodelijk G, de Jong M. Design and analysis of small-scale transmission experiments with animals. Epidemiology and Infection 2007;accepted for publication.
19 Donaldson AI. Risks of spreading foot and mouth disease through milk and dairy products. Rev Sci Tech 1997;16(1):117-24.
20 Graat EA, de Jong MC, Frankena K, Franken P. Modelling the effect of surveillance programmes on spread of bovine herpesvirus 1 between certified cattle herds. Vet Microbiol 2001;79(3):193-208.
21 Klinkenberg D, de Bree J, Laevens H, de Jong MC. Within- and between-pen transmission of Classical Swine Fever Virus: a new method to estimate the basic reproduction ratio from transmission experiments. Epidemiol Infect 2002;128(2):293-9.
22 Klinkenberg D, Everts-van der Wind A, Graat EA, de Jong MC. Quantification of the effect of control strategies on classical swine fever epidemics. Math Biosci 2003;186(2):145-73.