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Identification of factors associated with increased excretion of foot-and-mouth disease virus☆

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ABSTRACT

We investigated which variables possibly influence the amount of foot-and-mouth disease virus (FMDV) shed in secretions and excretions by FMDV infected animals, as it is likely that the amount of FMDV shed is related to transmission risk. First, in a separate analysis of laboratory data, we showed that the total amount of FMDV in secretions and excretions from infected animals is highly correlated with maximum titres of FMDV. Next, we collected data from 32 published scientific articles in which FMDV infection experiments were described. The maximum titres of FMDV reported in different secretions and excretions (the response variable) and the experimental conditions in which they occurred (the explanatory variables), were recorded in a database and analyzed using multivariate regression models with and without random effects. In both types of models, maximum titres of FMDV were significantly ($p < 0.05$) associated with types of secretions and excretions, animal species, stage of the disease and days post infection. These results can be used to prioritize biosecurity measures in contingency plans.

1. Introduction

Foot-and-mouth disease (FMD) is a contagious viral disease of cloven-hoofed animals, both domestic (cattle, pigs, sheep, goats and domestic buffalo) and wild (Thomson, 1994). The FMD virus (FMDV) can be transmitted by several routes (Sellers, 1971; Hyslop, 1970), with direct contact between animals considered the most important. The virus can also be transmitted by several indirect routes. In the European Union, an outbreak of FMD invokes an obligatory stand-still of animal transport (OIE, 2012). During such a stand-still, direct contact between infected animals in one farm and non-infected animals in another farm is theoretically impossible, leaving indirect transmission via contaminated material the most likely remaining route of transmission. In this respect, airborne transmission has been also considered (Henderson, 1969).

During epidemics, even when there is a complete standstill of animal transport, transmission between farms has been shown (Bouma et al., 2003). That indirect routes play a role in such transmission is clear from the observation that veterinarians were involved in the transmission of FMDV in outbreaks both in Denmark in 1982, and in Italy in 1993, either by using contaminated surgical equipment or by visiting farms after visiting an infected farm. Similarly, during the 2001 FMD outbreak in the United Kingdom, it was suggested that farmers were involved in the transmission of the virus between sheep flocks (Kitching, 2005). In the 2001 United Kingdom outbreak, the basic reproduction number remained above 1, that is, FMDV transmission continued despite the standstill in animal transport (Woolhouse et al., 2001). Thus indirect transmission of FMDV can have enormous consequences.

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It can be assumed that the risk of indirect transmission of FMDV is related to the total amount of FMDV present in the environment through contamination by secretions and excretions from FMDV infected animals. Here, secretions include material released from glands (e.g. milk, semen, saliva) whereas excretions refer to any other products released from animals (e.g. faeces, material released from the respiratory tract, urine, probang samples, nasal discharge and blood). The concentrations of FMDV in infected secretions and excretions have been reviewed (Pharo, 2002). However we analyzed the quantitative relationship between possible explanatory variables and the amount of FMDV in infected secretions and excretions.

2. Materials and methods

2.1. Materials

2.1.1. Laboratory data

Laboratory reports from animal studies performed at the Central Veterinary Institute (The Netherlands) were mined for all available daily data on virus secretion in milk from cattle (Orsel et al., 2007a) and on virus secretion and excretion in oropharyngeal fluid (OPF) swabs from cattle (Orsel et al., 2005), sheep (Eblé et al., 2008; Orsel et al., 2007b) and pigs (Eblé et al., 2004, 2006a,b, 2007; Orsel et al., 2007c). These data were used to identify the response variable for our multivariate regression analysis.

2.1.2. Literature data

Data on FMDV in secretions and excretions were collected from 32 scientific articles published between 1965 and 2007 (see Annex) found in internal databases and through the electronic (external) databases Scopus[1] and PubMed[2] in 2010, all reporting experimental trials involving FMDV infection. The electronic databases were explored using the keywords: foot-and-mouth disease, virus, infection and excretion. References cited in retrieved articles were reviewed to identify additional ones. The articles had to meet two basic criteria for their inclusion in the analysis: be written either in English, Spanish or French, and contain data on animal experiments with FMDV. They needed to contain information on the maximum titre(s) of FMDV detected in secretions and/or excretions, and additional information on one or more of the following: the type(s) of secretion or excretion in which the virus was detected, route of infection, animal species, FMDV serotype, stage of disease (clinical and non-clinical), dose of infection and/or days post infection at which the maximum secretion or excretion occurred. Missing data on one or more of these variables were recorded as not available (N.A.). These data were used as the response and possible explanatory variables for our multivariate regression analysis.

Per individual animal, the maximum titre of FMDV (including the experimental conditions) was recorded. Virus titres were reported as ^{10}log $TCID_{50}$/ml. Plaque forming units (PFU) were converted to $TCID_{50}$ (Horzinek, 1985). Median doses, such as 50% cattle infection dose (CID_{50}), 50% mouse infection dose (MID_{50}) or 50% mouse lethal dose (MID_{50}) per ml, were considered equal to 50% tissue culture infective dose ($TCID_{50}$/ml) (House and Yedloutschnig, 1982). The maximum recorded titre was the maximum titre over time for an individual animal. If the maximum titre was reported per group of animals, this resulted in one observation (from blood in Alexandersen et al. (2003); from airborne excretion in Alexandersen et al. (2002), Alexandersen and Donaldson (2002), Donaldson et al. (1970, 1981, 1982), Gloster et al. (2007), and Sellers and Parker (1969); from probang, milk, faeces and blood in Burrows (1968); from milk in Burrows (1971); and, from probang and nasal discharge in Burrows (1972)). Data on airborne excretion were recorded as ^{10}log $TCID_{50}$/animal/day.

The recorded secretion or excretion types were airborne, faeces, milk, probang, semen, urine, blood, nasal discharge, oropharyngeal fluid (OPF) swabs, and saliva. The category faeces contains data on material collected from the rectum (Burrows et al., 1968) and from rectal swabs (Garland, 1974). Probang refers to oropharyngeal samples that were obtained after scraping the oropharynx with a sampling cup.

Routes of infection were recorded as: contact (if an infected donor and a susceptible contact animal shared a common experimental unit); intranasal (IN, if the animals were infected via the intranasal route) or parenteral (if the animals were infected intravenously (IV), intramuscularly (IM), intralingually, intracutaneously (IC), intramammary or intradermally (ID)).

Animal species were recorded as cattle (bull, steer, ox, cow, calf and heifer), swine (pigs) or small ruminants (sheep, lambs and goats). The FMD viruses used for infection were recorded based on FMDV serotype, i.e. A, O, C, Asia 1, SAT 1, SAT 2, SAT 3, but no subdivision was made to the level of subtypes. The stage of disease was recorded as 'clinical' when lesions or clinical signs (including fever) were reported; otherwise it was recorded as 'non-clinical'.

Dose of infection (ranging from 0.95 to 10.15 $TCID_{50}$/ml) was recorded. Days post infection was recorded as the day when the maximum titres in the secretion or excretion were observed (ranging from 0.33 to 28 dpi).

2.2. Methods

2.2.1. Identifying the response variable for the multivariate regression analysis

A proxy for the total amount of FMDV secreted and excreted by the infected animals was established using available laboratory data from OPF swab samples and milk samples. The total amount of secreted and excreted FMDV (per individual animal) was calculated by summing the observed viral amounts (without logarithmic transformation) from consecutive observations (area under the curve, AUC). In a univariate regression analysis, the logarithm of the AUC (^{10}log AUC) was used as the response variable. Three explanatory variables were analyzed: (1) the maximum virus titre (max ^{10}log $TCID_{50}$/ml), (2) the time when the maximum virus titre occurred (^{10}log

[1] http://www.scopus.com/.
[2] http://www.ncbi.nlm.nih.gov/pubmed/.

Table 1A
Descriptive statistics on data retrieved from the literature on maximum virus excretion from cattle.

FMDV infection variables	Number of observations	Maximum titre average (range) $TCID_{50}/ml^*$	Maximum titre standard deviation $TCID_{50}/ml^*$
Total	220	4.51 (0.95,8.65)	1.66
Type of secretion and excretion			
Airborne	9	4.33 (3.88, 5.08)	0.36
Blood	47	4.03 (0.95,6.20)	1.18
Faeces	5	1.55 (1.50, 1.75)	0.10
Milk	40	4.48 (2.15, 7.35)	1.46
URT (OPF swabs, saliva and nasal discharge)	33	5.70 (1.25, 8.50)	1.66
Nasal discharge only	7	6.09 (2.75, 7.85)	1.61
Probang	68	4.91 (2.20, 8.65)	1.53
Semen	8	4.55 (2.10, 6.20)	1.33
Urine	10	1.93 (1.00, 3.80)	0.87
Route of infection			
Intranasal	37	4.68 (0.95, 8.65)	1.76
Parenteral	95	4.75 (1.25, 8.50)	1.63
Contact	88	4.17 (1.00, 8.05)	1.57
Undetermined	1	4.60 (NA)	NA
FMDV serotype			
A	38	3.98 (2.10, 8.05)	1.40
O	140	4.54 (0.95, 8.65)	1.68
Asia-1	4	4.10 (2.80, 5.00)	0.80
C	6	4.6 (2.10, 7.00)	1.80
SAT (1, 2, 3)	12	4.26 (2.10, 6.00)	1.06
Undetermined	20	5.52 (1.25, 8.15)	1.81
Stage of disease			
Non-clinical	61	4.52 (0.95, 8.65)	1.66
Clinical	123	4.62 (1.00, 8.50)	1.72
Undetermined	36	4.11 (1.15, 7.15)	1.36
Dose of infection (below/above median: 5.5 $TCID_{50}/ml$)			
0.95–5.4 $TCID_{50}/ml$	51	4.94 (0.95, 8.65)	1.71
5.5–10.15 $TCID_{50}/ml$	59	4.30 (2.10, 7.20)	1.48
Undetermined	110	4.43 (1.00, 8.15)	1.69
Days post infection (dpi; below/above median: 3 dpi)			
0.3–2.8 dpi	65	4.82 (1.00, 8.50)	1.69
3–28 dpi	115	4.07 (0.95, 8.65)	1.49
Undetermined	40	5.28 (1.25, 8.15)	1.67

Total refers to all the maximum titres observations that were encountered.

* $TCID_{50}$ per animal per day for airborne excretion; dose of infection and days post infection were divided as above and below the median of the maximum titre calculated using the maximum titres when either the dose of infection or the days post infection were available.

days post infection) and, (3) their product ^{10}log (max $TCID_{50}/ml \times$ days post infection) which is equal to max ^{10}log $TCID_{50}/ml + ^{10}$log days post infection. For each univariate model, the r^2 values were calculated. An *F*-test (in ANOVA) was used to test the significance of each variable. The best explanatory variable was used as response variable in the multivariate regression analysis.

2.2.2. Identifying the explanatory variables for the multivariate regression analysis

A dataset was built using the information found in the literature. Descriptive statistics of the data can be found in Tables 1A–1C.

Per individual animal, several categorical variables were recorded: type of secretion and excretion, route of infection, animal species, FMDV serotype and stage of disease and, two continuous variables: dose of infection and days post infection (Table 2). Categories in which a limited number of observations were present were combined with another category where this made biological sense (e.g. URT secretions and excretions, FMDV serotype SAT) (Dohoo et al., 2009).

2.2.3. Multivariate regression analysis

Under the assumption that all the included FMDV infection experiments share a common true effect size, we used a model in which we did not adjust for variability between data sources (a linear model without random effects). Under the assumption that some of the FMDV infection experiments from the different data sources differ from each other in ways that could impact on the effect in the model, we used a model in which we adjusted for variability between the data sources (a linear model with random effects). Three different random effects were evaluated: "article" (articles included in the analysis, see Annex), "laboratory" (laboratories where the original analyses had been performed) and their nested effect. All random effects were assumed to follow a Gaussian distribution (Ott and Longnecker, 2010). The models were compared by computing the AIC (Table 3).

Due to the small number of identified explanatory variables (Table 2), we used them all in the multivariate regression analysis of the models with and without random effects. To select the variables that best explained total FMDV secreted and excreted by infected animals, a stepwise regression procedure with bidirectional

Table 1B
Descriptive statistics on maximum virus excretion from swine.

FMDV infection variables	Number of observations	Maximum titre average (range) $TCID_{50}/ml^*$	Maximum titre standard deviation $TCID_{50}/ml^*$
Total	71	5.15 (3.41, 8.60)	0.98
Type of secretion and excretion			
Airborne	22	6.00 (4.48, 8.60)	0.89
Blood	6	5.18 (3.90, 6.50)	1.07
OPF (swabs and saliva)	43	4.70 (3.41, 6.45)	0.66
Route of infection			
Parenteral	39	5.44 (3.85, 8.08)	0.84
Contact	32	4.78 (3.41, 8.60)	1.01
FMDV serotype			
A	5	5.65 (4.48, 6.68)	0.70
O	64	5.01 (3.41, 6.54)	0.81
C	2	8.34 (8.08, 8.60)	0.26
Stage of disease			
Non-clinical	5	5.80 (5.30, 6.54)	0.50
Clinical	43	5.09 (3.41, 8.60)	1.04
Undetermined	23	5.11 (3.85, 8.08)	0.89
Dose of infection (below/above median: 5.5 $TCID_{50}/ml$)			
0.95–5.4 $TCID_{50}/ml$	19	4.94 (3.85, 6.50)	0.62
5.5–10.15 $TCID_{50}/ml$	18	6.01 (4.48, 8.10)	0.72
Undetermined	34	4.81 (3.41, 8.60)	0.99
Days post infection (dpi; below/above median: 3 dpi)			
0.3–2.8 dpi	22	5.59 (4.35, 8.60)	0.97
3–28 dpi	41	4.76 (3.41, 6.45)	0.77
Undetermined	8	5.95 (5.10, 8.10)	0.93

Total refers to all the maximum titres observations that were encountered.
* $TCID_{50}$ per animal per day for airborne excretion; dose of infection and days post infection were divided as above and below the median of the maximum titre calculated using the maximum titres when either the dose of infection or the days post infection were available.

Table 1C
Descriptive statistics on maximum virus excretion from small ruminants (sheep and goats).

FMDV infection variables	Number of observations average (range)	Maximum titre standard deviation $TCID_{50}/ml^*$	Maximum titre $TCID_{50}/ml^*$
Total	36	3.93 (0.86, 6.28)	1.25
Type of secretion and excretion			
Airborne	12	3.75 (2.38, 5.08)	1.00
Blood	8	3.34 (1.50, 5.20)	1.13
OPF (swabs and saliva)	16	4.37 (0.86, 6.28)	1.31
Route of infection			
Intranasal	11	4.69 (3.26, 6.28)	0.83
Parenteral	18	3.51 (1.50, 5.20)	1.10
Contact	6	3.70 (0.86, 5.45)	1.64
Undetermined	1	4.60 (NA)	NA
FMDV serotype			
A	2	2.53 (2.48, 2.58)	0.05
O	23	4.35 (0.86, 6.30)	1.12
C	3	3.28 (2.38, 5.08)	1.27
Undetermined	8	3.34 (1.50, 5.20)	1.13
Stage of disease			
Non-clinical	8	3.69 (0.86, 5.08)	1.37
Clinical	13	4.81 (3.26, 6.28)	0.79
Undetermined	15	3.30 (1.50, 5.20)	1.06
Dose of infection (below/above median: 5.5 $TCID_{50}/ml$)			
0.95–5.4 $TCID_{50}/ml$	12	4.69 (3.26, 6.28)	0.79
5.5–10.15 $TCID_{50}/ml$	7	3.33 (2.38, 5.08)	1.05
Undetermined	17	3.65 (0.86, 5.45)	1.33
Days post infection (dpi; below/above median: 3 dpi)			
0.3–2.8 dpi	20	3.74 (1.50, 5.26)	1.18
3–28 dpi	14	4.17 (0.86, 6.28)	1.34
Undetermined	2	4.23 (3.48, 4.98)	0.75

Total refers to all the maximum titres observations that were encountered.
* $TCID_{50}$ per animal per day for airborne excretion; dose of infection and days post infection were divided as above and below the median of the maximum titre calculated using the maximum titres when either the dose of infection or the days post infection were available.

Table 2
Explanatory variables for the multivariate regression analysis.

Variable	Type	Categories/specifications
Type of secretion and excretion	Categorical	Airborne, blood, faeces, milk, URT (OPF swabs, saliva, nasal discharge), probang, semen, urine
Route of infection	Categorical	Intranasal, contact, parenteral (intravenous, intramuscular, intralingual, intracutaneous, intramammary or intradermal)
Animal species	Categorical	Cattle, swine, small ruminants (sheep and goats)
FMDV serotype	Categorical	A, Asia-1, C, O, SAT
Stage of disease	Categorical	Non-clinical, clinical
Dose of infection	Continuous	From 0.95 to 10.15 $TCID_{50}$/ml
Days post infection (dpi)	Continuous	From day 0.33 to 28 post infection

elimination (Faraway, 2002) was used in multivariate regression analyses. No interaction terms were included in the initial (full) models (Table 3). The selection of explanatory variables (or fixed effects) was carried out using 2 criteria: the significance level ($p < 0.05$) and the Akaike Information Criterion (AIC). The variable with the highest p-value was removed from the models. In addition, whenever deletion of a variable occurred, we checked for confounding. If the deletion of a variable resulted in a change of more than 25% in the regression estimates, this indicated confounding (Hosmer and Lemeshow, 1989; Noordhuizen et al., 2001). Confounding variables were retained in the models. After deletion of those variables with p-values higher than 0.05, we tested whether their inclusion was significant ($p < 0.05$) and whether the inclusion led to significant reduction in AIC ($\Delta AIC > 2$, Burnham and Anderson, 2004). After selecting the explanatory variables of the models, one level interaction terms were included one by one in the models. When the interaction term allowed improvement of fit ($p < 0.05$), it remained in the models.

Both final models (Table 3) were checked for homoscedasticity, normality and outliers by residual analysis. Outliers were retained as they were thought to reflect relevant deviations in this sort of data. In order to test whether an outlier affected the estimates or the p-values, an outlier was excluded from the data and the models were re-fit. When the outlier had no influence on the estimates or p-values, it remained in the models.

All statistical analyses were performed using the R software version 2.11.0 with its standard add-on packages *stats* and *lme4* (R Development Core Team, 2012).

3. Results

3.1. Identifying the response variable for the multivariate regression analysis

The univariate regression analysis between ^{10}log AUC and max ^{10}log $TCID_{50}$/ml gave a correlation coefficient (r^2) of 0.98 for OPF swab samples and of 0.99 for milk samples (p-value <0.001). The analysis between ^{10}log AUC and ^{10}log days post infection gave correlation coefficients of 0.01 for OPF swab samples and 0.09 for milk samples. There was no significant association between ^{10}log AUC and ^{10}log days post infection (OPF swab samples, p-value 0.3; milk samples, p-value 0.2). The addition of ^{10}log days post infection in the model with max ^{10}log $TCID_{50}$/ml did not improve the fit of the model neither for OPF swab samples nor for milk samples (p-value 0.3 and 0.4 respectively). The variable max ^{10}log $TCID_{50}$/ml was therefore used as the response variable in the multivariate regression analysis.

Table 3
Comparison of fitted models for the max ^{10}log $TCID_{50}$/ml based on published data of FMDV infection studies using the same dataset (number of observations = 204).

	Explanatory variables	Interaction terms	Random effects	AIC
Model without random effects				
Null model	–	–	–	759.5
Full model	Type of secretion and excretion, dpi, animal species, route of infection, FMDV serotype, stage of disease	–	–	642.6
Final model	Type of secretion and excretion, dpi, animal species, FMDV serotype, stage of disease	Type of secretion and excretion*animal species, type of secretion and excretion*stage of disease, type of secretion and excretion*FMDV serotype, FMDV serotype*stage of disease	–	584.6
Model with random effects				
Null model	–	–	Article	727.4
			Laboratory	763.2
			Articles in laboratories	728.5
Full model	Type of secretion and excretion, dpi, animal species, route of infection, FMDV serotype, stage of disease	–	Article	622.7
Final model	Type of secretion and excretion, dpi, animal species, stage of disease	N.A.	Article	615.4

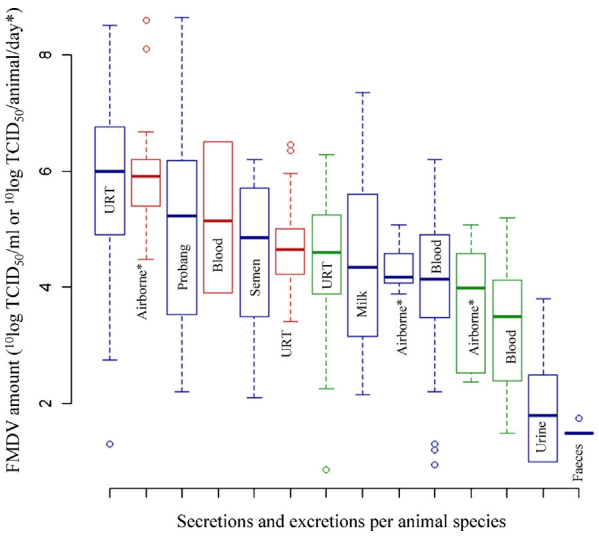

Fig. 1. Boxplot of FMDV amounts (^{10}log $TCID_{50}$/ml) in secretions and excretions from cattle (in dark blue), swine (in dark red) and small ruminants (in dark green). In airborne excretion (*), ^{10}log $TCID_{50}$/animal/day is reported. URT, upper respiratory tract secretions and excretions. When applicable, each column contains the extreme of the lower whisker, the lower hinge, the median, the upper hinge and the extreme of the upper whisker for one plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Literature data

The references of the 32 used scientific articles on FMDV infection experiments are shown in the Annex. The FMDV infection experiments reported in the selected scientific articles were carried out in 5 FMD reference laboratories: the Pirbright Institute (IAH, Pirbright, United Kingdom), the Plum Island Animal Disease Center (PIADC, Orient Point, New York, United States of America), the Central Veterinary Institute (CVI, Lelystad, The Netherlands), the Pan American Center for Foot-and-Mouth Disease (PanAftosa, Rio de Janeiro, Brazil) and the French Institute for Foot-and-Mouth Disease (Lyon, France).

In total 327 observations (220 cattle, 71 swine and 36 small ruminants) were retrieved. The data retrieved from the reviewed scientific articles are summarized in Table 1A for cattle, Table 1B for swine and Table 1C for small ruminants. All the observed maximum titres of FMDV in the different types of secretions and excretions per animal species were used to calculate the median maximum amounts and are shown in Fig. 1. The highest FMDV median amounts (^{10}log $TCID_{50}$/ml or ^{10}log $TCID_{50}$/animal/day) were found in URT secretions and excretions from cattle (OPF swabs, saliva and nasal discharge samples) followed by airborne excretion from swine, probang samples from cattle and blood from swine.

3.3. Identifying the explanatory variables for the multivariate regression analysis

Candidate explanatory variables for the multivariate regression analysis are shown in Table 2. Given that (1) OPF swabs and saliva are derived from the oral cavity, and (2) there were limited observations in the category nasal discharge (only available for cattle), we combined OPF swabs with saliva and with nasal discharge and called this upper respiratory tract secretions and excretions (URT). In Table 1A for cattle, we show both URT and nasal discharge separately to show that the ranges of the maximum titres of both are similar. Due to limited observations in the categories SAT 1, SAT 2 and SAT 3 from the categorical variable FMDV serotype, we also combined the categories SAT 1, SAT 2 and SAT 3 into the category SAT.

3.4. The final model

In total, data of 327 observations were used to identify which variables are associated to the amount of FMDV that is secreted and excreted by the infected animals. During the analysis, first we looked at the inclusion/exclusion of dose of infection because it had the highest *p*-value and because it's high number of missing data points (161). As the comparison of models can only be done between models with the same number of observations, we looked at the effect of dose of infection separately. Comparison of the full models (with 118 observations) with and without dose of infection for the data set where dose of infection was not missing revealed that the models without dose of infection had a lower AIC than the models with dose of infection. Therefore dose of infection was excluded from both full models. Subsequently, all the other variables were looked at (Table 3).

The final model without random effects is shown in Table 4. This model was fitted using 204 observations. Using the variables selection criteria (*p*-values and AIC), 4 explanatory variables were identified to be significantly associated with the total amount of FMDV secreted and excreted by infected animals: type of secretion and excretion, days post infection, stage of disease and FMDV serotype. Even though animal species had a *p*-value of 0.056, its inclusion improved the fit of the model (the AIC decreased), and its biological relevant. So, in total we identified 5 explanatory variables associated with the total amount of FMDV secreted and excreted by infected animals. No confounding factors were found. The explanatory variable route of infection dropped out during the stepwise regression procedure. In total 4 interactions terms were significantly associated with the total amount of FMDV secreted and excreted by infected animals: "type of secretion and excretion with animal species", "type of secretion and excretion with stage of disease", "type of secretion and excretion with FMDV serotype" and "FMDV serotype with stage of disease". Note that in Table 4 several combinations of categories could not be included in the interaction analysis because certain combinations of categories were not present in the used scientific articles (e.g. no information on amounts of FMDV in milk from swine could be retrieved from the scientific articles).

Airborne excretion, 0 dpi, cattle, clinical stage of disease and FMDV serotype A were chosen as reference categories. Compared to these reference categories, FMDV is found in higher quantities in probang samples (2.5 ^{10}log $TCID_{50}$/ml higher, *p*-value <0.001) and in lower quantities in

Table 4
Results of the final multivariate regression model. Reference categories: airborne, 0 dpi, cattle, clinical, A.

Variable	Category	Estimate	Std. error	*t*-value	*p*-value
Intercept	–	4.21	0.45	9.42	<2e−16
Explanatory variables					
Type of secretion and excretion	Blood	0.34	0.66	0.51	0.61
	Faeces	−2.29	0.72	−3.19	0.001
	Milk	−0.24	0.53	−0.45	0.65
	Urt	1.06	1.39	0.77	0.44
	Probang	2.50	0.73	3.43	<0.001
	Semen	−0.97	1.03	−0.94	0.35
	Urine	−1.90	1.03	−1.85	0.07
Days post infection	–	−0.07	0.02	−3.42	<0.001
Animal species	Small ruminants	−1.08	0.39	−2.77	0.01
	Swine	−1.58	0.34	−4.60	<0.001
Stage of disease	Non-clinical	−1.74	1.46	−1.19	0.24
FMDV serotype	Asia-1	0.70	1.31	0.53	0.59
	C	1.11	0.65	1.71	0.09
	O	−0.02	0.47	−0.05	0.96
	SAT	0.81	1.14	0.72	0.47
Interactions					
Type of secretion and excretion/animal species	Airborne: small ruminants	1.08	1.11	0.97	0.33
	Airborne: swine	3.62	0.58	6.26	<0.001
	Blood: swine	2.30	0.57	4.03	<0.001
Type of secretion and excretion/stage of disease	Blood: non-clinical	−0.97	1.37	−0.71	0.48
	Faeces: non-clinical	−0.70	1.70	−0.41	0.68
	Milk: non-clinical	1.83	1.63	1.12	0.26
	URT: non-clinical	−2.51	1.42	−1.77	0.08
	Probang: non-clinical	−0.64	1.35	−0.47	0.64
Type of secretion and excretion/FMDV serotype	Blood: Asia-1	−0.88	1.68	−0.52	0.60
	Semen: Asia-1	1.27	1.86	0.68	0.49
	Blood: C	−2.45	1.24	−1.98	0.05
	URT: C	0.58	1.61	0.36	0.72
	Semen: C	−2.11	1.47	−1.44	0.15
	Blood: O	0.12	0.71	0.17	0.86
	URT: O	1.00	1.42	0.71	0.48
	Probang: O	−0.46	0.77	−0.60	0.55
	Semen: O	2.81	1.17	2.40	0.02
	Urine: O	−0.55	1.11	−0.49	0.62
	Blood: SAT	−0.91	1.40	−0.65	0.52
	Semen: SAT	0.35	1.61	0.22	0.83
FMDV serotype/stage of disease	C: non-clinical	1.57	1.60	0.98	0.330
	O: non-clinical	2.18	0.65	3.35	0.001

faeces samples (2.3 ^{10}log TCID$_{50}$/ml lower, *p*-value 0.001). The quantity of secreted and excreted FMDV was high if the peak occurred soon after infection and decreased with time (0.07 ^{10}log TCID$_{50}$/ml decrease in time, *p*-value <0.001). The quantity of virus shed into the environment was also determined by animal species (e.g. cattle secrete and excrete FMDV in overall higher amounts than other animal species). Larger quantities of FMDV were associated with the presence of clinical signs. They were also associated with the FMDV serotype that initiated the infection (Table 4).

Based on the analysis of the interaction terms, the maximum amount of virus found in different secretions and excretions depends on the affected animal species, so a specific type of secretion or excretion from a particular animal species would have higher levels of FMDV than those from another animal species (e.g. airborne excretion from swine contain higher amounts of FMDV than airborne excretion from other species). For all secretions and excretions, except milk, the amount of FMDV was lower during the non-clinical stage than during the clinical stage. For milk it was about equal in the non-clinical and clinical stages.

The interaction term between type of secretion and excretion and FMDV serotype indicates that infection with some FMDV serotypes is associated with presence of more FMDV in a specific secretion or excretion. The interaction term between FMDV serotype and stage of disease indicates that during infection with a particular FMDV serotype, variations in the total amounts of secreted and excreted FMDV are seen during the non-clinical and clinical stages. The AIC of the final model without random effects was 584.6, the lowest AIC of the examined models (Table 3).

The final model with random effects is shown in Table 5. This model was fitted using 204 observations. Inclusion of the random effect "article" improved the fit of the model. In the model with random effects, 4 fixed effects (explanatory variables) were identified to be significantly associated with the total amount of FMDV released by the infected animals: type of secretion and excretion, animal species, stage of disease and days post infection. No confounding factors were found. The explanatory variables route of infection and FMDV serotype dropped out during the stepwise regression procedure. Because most of the possible interactions have to be estimated from comparisons

Table 5
Results of the final multivariate regression model with "article" as random effect. Reference categories: airborne, cattle, clinical, 0 dpi.

Variable	Category	Estimate	Std. error	*t*-value	*p*-value
Intercept	–	4.94	0.58	8.57	<2e−16
Explanatory variables					
Type of secretion and excretion	Blood	−0.76	0.63	−1.21	0.23
	Faeces	−3.55	0.79	−4.47	<0.001
	Milk	−1.11	0.71	−1.58	0.12
	URT	0.15	0.66	0.23	0.82
	Probang	1.05	0.64	1.64	0.10
	Semen	−0.91	0.73	−1.25	0.21
	Urine	−3.34	0.69	−4.84	<0.001
Animal species	Small ruminants	0.25	0.60	0.42	0.67
	Swine	1.33	0.43	3.13	0.002
Stage of disease	Non-clinical	−0.72	0.21	−3.33	0.001
Days post infection	–	−0.03	0.03	−1.26	0.21

between articles, we were only able to analyze the interaction terms in the model without random effects.

Airborne excretion, cattle, clinical stage of disease and 0 dpi were chosen as reference categories. Compared to these reference categories, FMDV is found in lower quantities in faeces samples (3.5 ^{10}log $TCID_{50}$/ml lower, p-value <0.001) and in urine (3.3 ^{10}log $TCID_{50}$/ml lower, p-value <0.001). The amounts of FMDV secreted and excreted into the environment are also determined by animal species (e.g. swine excrete higher amounts of FMDV by the airborne route than cattle, p-value = 0.002). It is also associated with the presence of clinical signs (i.e. in the non-clinical stage of the disease, animals secrete and excrete 0.72 ^{10}log $TCID_{50}$/ml less virus, p-value = 0.001). Further, the amounts of secreted and excreted FMDV are high when they occur early after infection and decrease when the peak occurs later in time. The AIC of the final model with random effects was 615.4 (Table 3).

Normality and homoscedasticity were violated neither in the model without random effects nor in the model with random effects, according to the residual analysis. One outlier (i.e. 8.1 ^{10}log $TCID_{50}$/ml from a probang sample from cattle; Burrows et al., 1981) was identified. The outlier was retained; excluding it from the analysis had no influence on the estimates or p-values.

4. Discussion

The aim of this study was to determine which variables influence the total amount of FMDV that is secreted and excreted by infected animals (expressed as the ^{10}log AUC). This study was performed because we assume that the risk of indirect transmission of FMDV is related to the total amount of FMDV present in the environment through contamination by secretions and excretions from FMDV infected animals.

The maximum titre of FMDV (max ^{10}log $TCID_{50}$/ml or, in the case of airborne excretion, max ^{10}log $TCID_{50}$/animal/day) showed a strong relation with the total amount of virus that is shed to the environment, expressed as the logarithm of the sum of consecutive daily observations on viral amounts (^{10}log AUC). The maximum titres can therefore be used as a proxy for the total amount of virus in excretions and secretions. FMDV maximum titres are reported in literature differently accordingly to the type of secretion or excretion; FMDV titres from airborne excretions are reported in ^{10}log $TCID_{50}$ per animal per day whereas FMDV titres from other types of the secretions and excretions are reported per 1 ml of sample (^{10}log $TCID_{50}$ per ml). In our study, the maximum titres, regardless of denominator (i.e. ^{10}log $TCID_{50}$/ml and ^{10}log $TCID_{50}$/animal/day) were treated similarly. However, during the interpretation of the results, the difference between denominators and the difference between the produced amounts of secretions and excretions have to be taken into account (note that infected cows can produce several litres of contaminated milk per day).

The method used in this study allowed estimation of the effect of variables on our variable of interest: the maximum virus titre. One of the advantages of this method is its ability to bring together lots of information from numerous studies on animal experiments with FMDV without the need to perform new animal experiments. During the analysis of the model with random effects, we found that the random effect "article", possibly more accurately named "specific experimental conditions", influences the outcome of the model. We therefore report two models, a model without random effects and a model with "article" as a random effect. Both models identified the same explanatory variables except for FMDV serotype, but the latter could be explained due to the high correlation between FMDV serotype and the source of the data (i.e. FMDV serotype O was used in 27 articles and most of the analyzed articles report the use of only one FMDV serotype). In addition, we were unable to analyze the interaction terms in the model with random effects. But because the two final models contain almost the same variables, we reported also the results of the analysis of the interaction terms of the model without random effects. Moreover, the model without random effects including interaction terms had the lowest AIC. Furthermore, we consider the interaction terms biologically relevant.

The interaction between "type of secretion and excretion" and "animal species" shows that animal species influences the relation between the maximum titres of FMDV and the "type of secretion and excretion", with types of secretion and excretion linked to particular species. For the airborne route, as previous research shows, more FMDV is excreted by swine (Donaldson et al., 1970, 1982; Sellers and Parker, 1969). For other routes, as has been

mentioned, the major secretors and excreters of the virus are cattle (Thomson, 1994). The latter has been confirmed by our results; our dataset (Fig. 1) shows that URT secretions and excretions from cattle can contain very high amounts of FMDV (virus titres per ml), in some cases even higher than the amounts that are contained in airborne excretions from swine (virus titres per day). Considering that one of the clinical signs of FMD in cattle is profuse salivation, large amounts of saliva with FMDV can be found on the floor of an infected farm, making the contamination of different farm appliances (e.g. feedstuff, boots, veterinary appliances) feasible and therefore it could be an important vehicle for transmission of the virus between farms. In addition, even though milk production drops after infection with FMDV, an infected cow still produces 12–16 litres of milk per day (Orsel et al., 2007a) meaning that the total amount of secreted virus with milk is 4 ^{10}log higher than depicted in Fig. 1, much higher than the amount in airborne excretion from swine (Fig. 1). So the concern about dispersal of FMDV between farms by the bulk tankers is realistic (Thomson, 1994).

Beside the interaction between "type of secretion and excretion" and "animal species" our analysis showed that also the interaction between "type of secretion and excretion" and "stage of disease" was significant. In general, the amounts of FMDV are higher in clinically diseased animals but this is not the case in milk, where it is about the same, with high amounts of FMDV reported in milk samples when clinical signs were not apparent (see Burrows et al., 1968 referring to milk; Hyslop, 1965 referring to saliva). While the risk of transmission has been considered (see Charleston et al., 2011) low in the early stage of infection before clinical signs are apparent, it has also been shown that the basic reproduction number (the average number of new infections cause by a typical infectious individual in a totally susceptible population) is above 1, meaning that major outbreaks can still occur in pre-clinical dairy cows and pigs (Orsel et al., 2009). In the study by Charleston et al. (2011) it was shown that the calves were not infectious until on average 0.5 days after clinical signs appeared, even though FMDV was detected in secretions and excretions before the appearance of clinical signs. But, in contrast to the study of Orsel, the contact time between calves in the Charleston study was limited to only 8 h. This could explain why they did not observe transmission between calves before clinical signs appeared. Further, one should realize that FMD clinical signs are in some cases difficult to detect (see Donaldson and Sellers, 2000 on sheep, and Kitching and Hughes, 2002 on sheep and goats).

The last two interactions, i.e. between "type of secretion and excretion" and "FMDV serotype" and between "FMDV serotype" and "stage of disease" show that FMDV serotype influences both the relation between maximum titre and type of secretion and excretion and the relation between maximum titre and stage of disease. Similar FMDV serotype-dependent differences have been described for FMDV elsewhere (Kitching, 2005). Moreover, infection with FMDV serotype O may also lead to higher secretion and excretion of the virus during the non-clinical stage of disease. However, when adjusting for variability between experimental conditions (when using "article" as random effect), we found that FMDV serotype is highly correlated to the source of the data. Therefore, conclusions on FMDV serotype must be taken carefully.

Summarizing, we show that the total amount of FMDV secreted and excreted by infected animals depends mainly on the maximum titres of FMDV. Secondly, we have identified variables related to the maximum amount of secreted and excreted FMDV. To relate our findings with the risk of transmission of FMDV, future research will need to quantify the FMDV-contaminated material transported between farms and determine the infection rates from this contaminated material. The outcome of this analysis shows which secretion(s) and/or excretion(s) are of major risk for contaminating the environment with FMDV. These results can be used to prioritize biosecurity measures in contingency plans.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Annex. References of the articles included in the analysis

[1] Alexandersen S., Donaldson A.I., 2002. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. Epidemiol. Infect. 128, 313–323.
[2] Alexandersen S., Oleksiewicz M.B., Donaldson A.I., 2001. The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT-PCR. J. Gen. Virol. 82, 747–755.
[3] Alexandersen S., Zhang Z., Reid S.M., Hutchings G.H., Donaldson A.I., 2002. 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. 83, 1915–1923.
[4] Alexandersen S., Quan M., Murphy C., Knight J., Zhang Z., 2003. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. J. Comp. Pathol. 129, 268–282.
[5] Augé de Mello P., Sutmoller P., 1977. Observaciones preliminares sobre la replicación del virus en la faringe y viremia después de inoculación intradermolingual en bovinos con virus de la fiebre aftosa. Bltn Centro Panamericano Fiebre Aftosa 27–28, 13–17.
[6] Blackwell J.H., McKercher P.D., Kosikowski F.V., Carmichael L.E., Gorewit R.C., 1982. Concentration of foot-and-mouth disease virus in milk of cows infected under simulated field conditions. J. Dairy Sci. 65, 1624–1631.

[7] Burrows R., 1968a. Excretion of foot-and-mouth disease virus prior to the development of lesions. Vet Rec, 387–388.
[8] Burrows R., 1968b. The persistence of foot-and-mouth disease virus in sheep. J. Hyg. 66, 633–640.
[9] Burrows R., 1972. Early stages of virus infection: studies in vivo and in vitro. In: Symposia of the society for general microbiology 22, 303–332.
[10] Burrows R., Mann J.A., Greig A., Chapman W.G., Goodridge D., 1971. The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. J. Hyg. 69, 307–321.
[11] Burrows R., Mann J.A., Garland A.J., Greig A., Goodridge D., 1981. The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle. J Comp Pathol. 91, 599–609.
[12] Cottral, P.G., 1971. Experimental multiple infection of animals with foot-and-mouth disease viruses. In: Proc. Annu. Meet. US Anim. Health Assoc., 441–465.
[13] Cottral G.E., Bachrach H.L., 1968. Food-and-mouth disease viremia. In: Proc. Annu. Meet. US Anim. Health Assoc. 72, 383–399.
[14] Cottral G.E., Gailiunas P., Cox B.F., 1968. Foot-and-mouth disease virus in semen of bulls and its transmission by artificial insemination. Archiv. für die gesamte Virusforschung 23, 362–377.
[15] Cunliffe H.R., Blackwell J.H., 1977. Survival of foot-and-mouth disease virus in casein and sodium caseinate produced from the milk of infected cows. J. Food Prot. 40, 389–392.
[16] de Leeuw P.W., van Bekkum J.G., Tiessink J.W., 1978. Excretion of foot-and-mouth disease virus in oesophageal–pharyngeal fluid and milk of cattle after intranasal infection. J. Hyg. 81, 415–425.
[17] Donaldson A.I., Herniman K.A., Parker J., Sellers R.F., 1970. Further investigations on the airborne excretion of foot-and-mouth disease virus. J. Hyg. 68, 557–564.
[18] Donaldson A.I., Garland A.J., Ferris N.P., Collen T., 1981. The 1975 Foot-and-Mouth disease epidemic in Malta. I: Experimental studies with the causal virus strain O1 Malta. Br. Vet. J. 137, 300–307.
[19] Donaldson A.I., Ferris N.P., Gloster J., 1982. Air sampling of pigs infected with foot-and-mouth disease virus: comparison of Litton and cyclone samplers. Res. Vet. Sci. 33, 384–385.
[20] Eblé P.L., Bouma A., de Bruin M.G., van Hemert-Kluitenberg F., van Oirschot J.T., Dekker A., 2004. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. Vaccine 22, 1372–1378.
[21] Eblé P.L., Bouma A., Weerdmeester K., Stegeman J.A., Dekker A., 2007. Serological and mucosal immune responses after vaccination and infection with FMDV in pigs. Vaccine 25, 1043–1054.
[22] Fontaine J., Bornarel P., Dubouclard C., Stellmann C., Lang R., 1969. Anti-aphthous vaccination of sheep. Bull. Off. Int. Epizoot. 71, 421–442.
[23] Garland A.J., 1974. The inhibitory activity of secretions in cattle against FMDV (PhD thesis). London School of Tropical Hygiene. University of London and The Virus Animal Research Institute. Pirbright, Surrey, United Kingdom.
[24] Gloster J., Williams P., Doel C., Esteves I., Coe H., Valarcher J.F., 2007. Foot-and-mouth disease quantification and size distribution of airborne particles emitted by healthy and infected pigs. Vet. J. 174, 42–53.
[25] Hyslop N., 1965. Secretion of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle. J. Comp. Pathol. 75, 111–117.
[26] McVicar J.W., Sutmoller P., 1976. Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation. J. Hyg. 76, 467–481.
[27] Orsel K., de Jong M.C., Bouma A., Stegeman J.A., Dekker A., 2007a. Foot-and-mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine 25, 6381–6391.
[28] Orsel K., de Jong M.C., Bouma A., Stegeman J.A., Dekker A., 2007b. The effect of vaccination on foot-and-mouth disease virus transmission among dairy cows. Vaccine 25, 327–335.
[29] Orsel K., Dekker A., Bouma A., Stegeman J.A., de Jong M.C., 2007c. Quantification of foot-and-mouth disease virus excretion and transmission within groups of lambs with and without vaccination. Vaccine 25, 2673–2679.
[30] Scott F.W., Cottral. G.E., Gailiunas., P., 1966. Persistence of foot-and-mouth disease virus in external lesions and saliva of experimentally infected cattle. Am. J. Vet. Res. 27, 1531–1536.
[31] Sellers R., Burrows R., Mann J., Dawe P., 1968. Recovery of virus from bulls affected with foot-and-mouth disease. Vet. Rec. 83, 303.
[32] Sellers R.F., Parker J., 1969. Airborne excretion of foot-and-mouth disease virus. J. Hyg. 67, 671–677.

References

Bouma, A., Elbers, A.R., Dekker, A., de Koeijer, A., Bartels, C., Vellema, P., van der Wal, P., van Rooij, E.M., Pluimers, F.H., de Jong, M.C., 2003. The foot-and-mouth disease epidemic in The Netherlands in 2001. Prev. Vet. Med. 57, 155–166.
Burnham, K.P., Anderson, D.R., 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociol. Methods Res. 33, 261–304.
Charleston, B., Bankowski, B.M., Gubbins, S., Chase-Topping, M.E., Schley, D., Howey, R., Barnett, P.V., Gibson, D., Juleff, N.D., Woolhouse, M.E., 2011. Relationship between clinical signs and transmission of an infectious disease and the implications for control. Science 332, 726–729.
Dohoo, I., Martin, W., Stryhn, H. (Eds.), 2009. Veterinary Epidemiologic Research. VER Inc., Canada.
Donaldson, A.I., Sellers, R.F. (Eds.), 2000. Foot-and-Mouth Disease. Blackwell Science, Oxford.
Eblé, P.L., Bouma, A., De Bruin, M.G., van Hemert-Kluitenberg, F., van Oirschot, J.T., Dekker, A., 2004. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. Vaccine 22, 1372–1378.
Eblé, P., de Koeijer, A., Bouma, A., Stegeman, A., Dekker, A., 2006a. Quantification of within- and between-pen transmission of Foot-and-Mouth disease virus in pigs. Vet. Res. 37, 647–654.
Eblé, P.L., De Bruin, M.G., Bouma, A., Van Hemert-Kluitenberg, F., Dekker, A., 2006b. Comparison of immune responses after intra-typic heterologous and homologous vaccination against foot-and-mouth disease virus infection in pigs. Vaccine 24, 1274–1281.
Eblé, P.L., Bouma, A., Weerdmeester, K., Stegeman, J.A., Dekker, A., 2007. Serological and mucosal immune responses after vaccination and infection with FMDV in pigs. Vaccine 25, 1043–1054.
Eblé, P.L., Orsel, K., Dekker, A., 2008. Quantification of transmission of FMDV strain Asia-1 Turkey among vaccinated and non-vaccinated

lambs. In: Session of the Research Group of the European Commission for the Control of Foot-and-Mouth Disease, Erice, Sicily, Italy.
Faraway, J.J., 2002. Practical Regression and ANOVA using R (online) http://cran.r-project.org/doc/contrib/Faraway-PRA.pdf (consulted 2011).
Henderson, R.J., 1969. The outbreak of foot-and-mouth disease in Worcestershire. An epidemiological study; with special reference to spread of disease by wind carriage of the virus. J. Hyg. Camb. 67, 21.
Horzinek, M.C. (Ed.), 1985. Het virus als deeltje: Methodiek van het virusonderzoek. Algemene Virology: een compendium. Wetenschappelijke Uitgeverij Bunge, Utrecht, The Netherlands.
Hosmer, D., Lemeshow, S. (Eds.), 1989. Applied Logistic Regression. Wiley, New York.
House, J.A., Yedloutschnig, R.J., 1982. Sensitivity of seven different types of cell cultures to three serotypes of foot-and-mouth disease virus. Can. J. Comp. Med. 46, 186–189.
Hyslop, N., 1965. Secretion of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle. J. Comp. Pathol. 75, 111–117.
Hyslop, N., 1970. The epizootiology and epidemiology of foot-and-mouth disease. Adv. Vet. Sci. 14, 261–307.
Kitching, R.P., Hughes, G.J., 2002. Clinical variation in foot-and-mouth disease: sheep and goats. Rev. Sci. Tech. 21, 505–512.
Kitching, R.P., 2005. Global epidemiology and prospects for control of foot-and-mouth disease. Curr. Top. Microbiol. Immunol. 288, 133–148.
Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V., Graat, E.A.M. (Eds.), 2001. Application of Quantitative Methods in Veterinary Epidemiology. Wageningen Pers Publ, Wageningen, The Netherlands.
OIE: World Organization for Animal Health, 2012. Foot-and-Mouth Disease, Available from: http://www.oie.int/eng/A_FMD2012/docs/en_chapitre_1.8.5.pdf (accessed 13.06.13).
Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A., De Jong, M.C., 2005. Vaccination against foot-and-mouth disease reduces virus transmission in groups of calves. Vaccine 23, 4887–4894.
Orsel, K., De Jong, M.C., Bouma, A., Stegeman, J.A., Dekker, A., 2007a. The effect of vaccination on foot-and-mouth disease virus transmission among dairy cows. Vaccine 25, 327–335.
Orsel, K., Dekker, A., Bouma, A., Stegeman, J.A., De Jong, M.C., 2007b. Quantification of foot-and-mouth disease virus excretion and transmission within groups of lambs with and without vaccination. Vaccine 25, 2673–2679.
Orsel, K., De Jong, M.C., Bouma, A., Stegeman, J.A., Dekker, A., 2007c. Foot-and-mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine 25, 6381–6391.
Orsel, K., Bouma, A., Dekker, A., Stegeman, J.A., De Jong, M.C., 2009. Foot-and-mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows. Prev. Vet. Med. 88, 158–163.
Ott, R.L., Longnecker, M., 2010. An Introduction to Statistical Methods and Data Analysis, sixth ed. Brooks/Cole, Cengage Learning, Canada, pp. 1044.
Pharo, H.J., 2002. Foot-and-mouth disease: an assessment of the risks facing New Zealand. N. Z. Vet. J. 50, 46–55.
R Development Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL: http://www.R-project.org/
Sellers, R.F., Parker, J., 1969. Airborne excretion of foot-and-mouth disease virus. J. Hyg. 67, 671–677.
Sellers, R.F., 1971. Quantitative aspects of the spread of foot-and-mouth disease. Vet. Bull. 41, 431–439.
Thomson, G.R., 1994. Foot-and-mouth disease. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (Eds.), Infectious Diseases of Livestock with Special Reference to Southern Africa. Oxford University Press Southern Africa, Capetown, pp. 825–851.
Woolhouse, M., Chase Topping, M., Haydon, D., Friar, J., Matthews, L., Hughes, G., Shaw, D., Wilesmith, J., Donaldson, A., Cornell, S., Keeling, M., Grenfell, B., 2001. Epidemiology foot-and-mouth disease under control in the UK. Nature 411, 258–259.