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**Comparison of viraemia- and clinical-based estimates of within- and
between pen transmission of Classical Swine Fever Virus from three
transmission experiments.**

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Abstract.

Analyses of recent classical swine fever (CSF) epidemics in the European Union have shown that silent circulation of CSF virus (CSFV) occurs before the first outbreak is detected and this may lead to a large epidemic. However, severity of CSF disease signs may be linked with efficacy of disease transmission, the most severely affected animals having a higher infectivity than the less affected ones. The purpose of this study was to combine disease transmission quantification methods with CSF clinical signs quantification tools to investigate whether clinical signs, considered as infectivity markers, may allow us to calculate reliable estimates for disease transmission parameters. Data from three transmission experiments were used, varying according to the viral strain (Eystруп or Paderborn) and to the contact structure between experimentally inoculated and contact animals (direct or indirect contact). Within- and between-pen basic reproduction ratios (R_0) were compared using viraemia data or clinical data. Between-pen R_0 estimates were close and not significantly >1 , with either strain or computation mode (using viraemia or clinical data). Conversely, within-pen R_0 s (Paderborn strain) computed using clinical data appeared higher than the estimates obtained using viraemia data. A models comparison (Bayes information criterion) showed a better fit of the clinical-based models, for both strains. This suggests that, in affected herds, the most severely affected animals could play a prominent role in CSFV transmission.

Keywords. classical swine fever, pig, basic reproduction ratio, clinical score

1. Introduction

Classical swine fever (CSF) is a highly contagious viral disease of worldwide importance. Domestic and feral pigs are the only natural reservoir of Classical Swine Fever Virus (CSFV). The disease is endemic in many countries of Eastern Europe, South-Eastern Asia, and Southern and Central America (Dahle and Liess 1992, Moennig 2000). When introduced in a fully susceptible population, CSFV may cause large epidemics. In 1980 the European Union (EU) adopted an eradication strategy in pig herds, based upon a stamping-out policy. Initially this policy involved mass vaccination but, from 1992 onwards was implemented without preventive vaccination. However, emergency vaccination was still possible in large outbreaks. In many EU countries currently, CSF has been eradicated from the domestic pig population (Anon., 2007) but it remains endemic in some wild boar populations, posing a risk of outbreaks on farms (Fritzemaier et al. 2000, Fröhlich et al. 2002).

CSF outbreaks cause huge economic losses and have important consequences for the pig industry, especially in densely populated areas where the virus may spread quickly (Meuwissen et al. 1999, Saatkamp et al. 2000). Early detection of CSFV circulation is therefore crucial for minimizing epidemic size and economic consequences. Recent outbreaks have shown how a prolonged silent circulation of CSFV in pig herds may result in large epidemics (Elbers et al. 1999). Vague and non-specific disease symptoms may allow virus to spread unnoticed (Koenen et al. 1996, Sandvik et al. 2000, Terpstra and De Smit 2000). Severity of clinical signs is known to vary according to the CSFV strain, but also according to several other factors such as age, breed, health and immune status (Depner et al. 1997, Floegel-Niesmann et al. 2003, Moennig et al. 2003). Subacute or chronic disease courses observed in recent outbreaks pose real diagnostic problems (Elbers et al. 2004, Engel et al. 2005). However, the severity of clinical signs is probably linked with disease transmission.

The most severely affected animals are also probably the most contagious animals, as suspected for other pig diseases such as foot and mouth disease (Alexandersen et al. 2003, Quan et al. 2004). There is also a correlation between virus growth in cell culture and severity of disease caused. *In vitro* results show the amount of virus released in cell culture media is higher when cells are infected with strains causing more severe clinical signs compared to those causing less severe disease (Mittelholzer et al. 2000). Thus less severe disease forms could be associated with a lower disease transmission and a slower CSFV spreading in cells.

Few studies have been conducted to quantify the severity of CSF clinical signs. A first clinical scoring system was developed by Wood et al. (1988) to quantify the virulence of a specific strain. More recently, Mittelholzer et al. (2000) proposed a general and practical scoring system based upon a semi-quantitative judgement of 10 CSF-relevant criteria using scores varying from 0 to 3. They used this score to compare the clinical signs provoked by several CSFV isolates in SPF animals. Floegel-Niesman et al. (2003) added to these clinical scores a pathological score to quantify lesions, and used both scores to compare recent CSFV isolates, with isolates obtained during the 1990's.

The basic reproduction ratio (R_0) is the most widely used parameter to quantify the transmission of an infection. It is defined as the average number of secondary infected individuals caused by a single typical infectious individual in an infinite susceptible population (Anderson and May 1991). If R_0 is smaller than 1, an infectious animal infects, on average, less than one susceptible animal and the infection process wanes. Conversely if R_0 is greater than 1, the infection spreads, and a large epidemic can occur. Methods designed to estimate R_0 from experimental transmission data are either based on the sole final state of the animals (infected or not) (Kroese and De Jong 2001) or on a reconstruction of the within-group contagion process. In this method for each animal, an infection date and a period during which the animal has been infectious are computed (Becker 1989, De Jong and Kimman

1994, Klinkenberg et al. 2002). Several CSF R_0 estimates based upon transmission experiments have been published (Dewulf et al. 2002, Laevens et al. 1998, Laevens et al. 1999, Dewulf et al. 2001). In these the within-group contagion process was reconstructed using results of CSFV isolation from blood: an animal was considered as infectious as long as it was viraemic, and individual infection dates were computed assuming a latent period (between infection and the beginning of viraemia) of fixed duration. These were either estimated from values observed in experimentally inoculated animals (Laevens et al. 1998 and 1999), or fixed as a parameter (Klinkenberg et al. 2002).

The objective of this study was to investigate whether CSFV clinical signs, considered as infectivity markers, may enable reliable estimates of disease transmission parameters to be estimated.

We compared the R_0 estimates based on viraemia, with the values obtained by quantification of the clinical signs using the scoring system proposed by Mittelholzer et al. (2000). We used data from three transmission experiments conducted in 2001 using two strains with different virulence levels. Method- and strain-specific R_0 s estimates were calculated, and the differences are discussed.

2. Materials and Methods

2.1. Cells and virus

The highly-virulent Eystrup strain, genetic subtype 1.1 was kindly provided by A. Summerfield, Institute of Virology and Immunoprophylaxis, Mitterhäusern, Switzerland; with the permission of H.J. Thiel, Institute of Virology, University of Giessen, Germany. The moderately virulent Paderborn strain (CSF0277), genetic subtype 2.1, was kindly provided by the Community Reference laboratory (CRL) for CSF, Hanover, Germany). The strains were

propagated twice on PK15 cells (obtained from the CRL, Hanover, Germany) as recommended (Anon., 2002).

2.2. Transmission experiments

Experimental procedures and animal management were undertaken according to the French legislation on animal experimentation (registration number 22-17).

Three transmission experiments were conducted in 2001 in the Level 3 protected facilities at AFSSA -Ploufragan, using two different CSFV strains. The Eystrup strain was used in an indirect transmission experiment (A) whereas the Paderborn strain was used both in an indirect (B) and a direct contact transmission experiment (C).

Eight week old specific pathogen free (SPF) and pestivirus-free Large-White pigs weighing 35 kg and originating from the AFSSA protected facilities of Ploufragan were used. Experimentally inoculated animals received an oronasal dose of 10^6 TCID₅₀ (tissue culture 50% infective dose) CSFV (2 ml per nostril). Control pigs received 4 mls of culture medium oronasally instead of virus.

Two different experimental designs were used, with two pens (numbered 1 and 2). In experiments A and B, 5 experimentally inoculated pigs were placed in pen 1, and 4 contact pigs in pen 2. Both pens were physically separated, and animal care was organized so that only airborne transmission between infected and non-infected animals could occur. In experiment C, 2 experimentally inoculated pigs and 3 contact pigs were placed in each pen. Separation between pens was removed to allow direct contacts between animals. The three experiments thus differed according to the virus strain and to the studied transmission mode. In experiment A: Eystrup strain was used to study between-pen transmission. In experiment B: Paderborn strain was used to study between-pen transmission, and in experiment C: Paderborn strain was used to study within-pen transmission. As the design for experiment C

allowed direct contacts between the two pens, data from this experiment were treated as if all of the 10 animals had shared the same pen.

Pigs were examined daily using the clinical scoring system proposed by Mittelholzer et al. (2000). This consists of 10 criteria scored from 0 to 3 according to the severity of the symptoms (0: no symptoms, 3: severe symptoms). Scores are added to obtain an individual clinical score (CS). Of the 10 criteria proposed by Mittelholzer et al. (2000), the 10th (leftovers in feeding troughs) could not be determined, since in our facilities the animals are fed *ad libitum* in a common trough. The measured CSs thus ranged from 0 to 27, instead of 0 to 30.

Blood samples were taken from the jugular vein in heparin sterile tubes before the experimental inoculation (day 0), and on days 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28 (experiment A); 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28, 31, 33, 35, 38, 40, 42 (experiment B); and 3, 7, 9, 11, 14, 16, 18, 12, 23, 25, 27, 28, 31, 35, 38, 45 (experiment C). Samples were missing for 3 pigs on D24 (animals 6903, 6884, 6909) and for one animal on D33 (pig 6886). Blood was inoculated into wells containing confluent PK15 cell monolayers. After two passages, the supernatants were removed and the cells were fixed with cold acetone (80%). Diluted pig anti-CSFV antibodies followed by a mouse FITC conjugate were added to identify virus presence by fluorescence microscopy (Anon. 2002).

Pigs were followed-up (clinical scores and CSFV isolation from blood) until the disappearance of the clinical signs and the end of the viraemic period at day 28 (experiment A), 42 (experiment B) and 45 (experiment C). All the pigs were euthanised by intravenous booster of sodium pentobarbital at the end of the experiment or earlier if needed for welfare reasons.

2.3. *Virological- and clinical-based datasets*

For each of the three experiments, three different methods were used to reconstruct the contagion process from raw data, resulting in three different datasets: a viraemia-based dataset and two clinical-based datasets.

The viraemia-based dataset was analogous to the datasets used in previous studies (Laevens et al. 1998 and 1999, Klinkenberg et al., 2002). Missing data (days without CSFV isolation assay) were first linearly interpolated from the nearest observations. Infection dates were then calculated from the date of the 1st CSFV isolation from blood, assuming a latent period (between infection and the 1st CSFV isolation from blood) of fixed duration. This latent period was estimated from the average duration between infection and the 1st CSFV isolation from blood observed in experimentally inoculated animals. Animal infectivity was also based upon results of CSFV isolation from blood, an animal being considered as infectious as long as it was viraemic.

The two clinical-based datasets were derived from the CSs. In both cases, infection dates were based upon the 1st day with a CS>0, using again a fixed-length latent period (between infection and the 1st day with CS>0). The duration of this latent period was estimated from the average value observed in experimentally inoculated animals. The two clinical-based datasets differed according to how individual infectivity was derived from the clinical signs. These treated CSs as a quantitative or as a qualitative variable. For the quantitative clinical-based dataset, animal infectivity was directly based on CSs: at a given time point, the infectivity of an animal was assumed to be measured by its CS. Conversely, for the qualitative clinical-based dataset, an animal was considered as infectious as long as its CS was >0.

Whatever the dataset, the duration of the latent period was assumed to be strain-specific and was thus calculated once for the Eystrup strain (using data from experimentally

inoculated animals, experiment A) and once for the Paderborn strain (using data from experimentally inoculated animals, experiments B and C).

For each experiment, pen and time step, the following variables were calculated: the number of animals and the number of uninfected animals in the pen at the beginning of the time step, the number of contact animals infected during the time step, and the infectivity released by animals located in the pen, during the time step. For the viraemia-based dataset this infectivity was modelled by the number of viraemic animals in the pen, for the qualitative clinical-based dataset it was the number of animals in the pen presenting a CS>0, and for the quantitative clinical-based dataset it was the sum of their CSs.

2.4. Basic reproduction ratio estimation

The R0 estimation method was adapted from Klinkenberg et al (2002). This method is based upon a SIR (Susceptible-Infected-Removed) model [2] of which α and β parameters are estimated separately. The α parameter is the recovery rate of infectious animals, $1/\alpha$ is thus the average duration of the infectious period and, more generally, $1/\alpha$ represents the average total infectivity released by an infected animal. The β parameter is the contact rate between animals, split here in two parameters: the within- and between-pen contact rates, denoted respectively β_w and β_b . The within- and between-pen R0s are then, respectively: $R0_w = \beta_w / \alpha$, and $R0_b = \beta_b / \alpha$.

The β s were estimated as described by Klinkenberg et al. (2002): a maximum likelihood method was used, based upon a binomial distribution of the number C of animals being contaminated in a given pen during a given time step:

$$C \sim B[S, 1 - \exp(-(\beta_w I + \beta_b J) / N)], \text{ where:}$$

N and S are respectively the number of animals and the number of non-contaminated animals in the pen at the beginning of the time step;

$\mathcal{I}$ and $\mathcal{J}$ denote the total infectivity released during the time step by animals located in the same pen ($\mathcal{I}$) and by animals located in the neighbouring pen ($\mathcal{J}$);

$B[n, p]$ is the binomial distribution of parameters n and p .

To estimate α , Klinkenberg et al. (2002) used a general linear model for survival analysis with censoring. This approach is adopted when an animal is considered as either infectious or not. The duration of the infectious period can then be modelled using survival analysis techniques. However, this is not the case when infectivity is not a binary value such as a viraemic status, but a numeric quantity such as a CS. Due to this and the fact that there was no censored data because animals were followed-up until the end of clinical signs and of viraemia, we used a simple non-parametric estimate of $1/\alpha$: the average total infectivity released by an individual over the course of the infection. For the viraemia-based dataset, the infectious period was the average duration of viraemia. The point estimate of $1/\alpha$ (and thus of the R_0 s) is then computed as in Klinkenberg et al. (2002) assuming uncensored data. For the clinical-based dataset, it was either the average total CSs observed in infected animals (quantitative dataset) or the average number of days with a CS>0 (qualitative dataset).

2.5. Model comparison

For the Eystrup strain, only the between-pen R_0 was estimated (from experiment A data). For the Paderborn strain both R_0 s were estimated using data from experiments B (between-pen transmission) and C (within-pen transmission). Eystrup and Paderborn R_0 s estimates were computed separately from the virological- and clinical-based datasets.

The models comparison was based upon the Bayes Information Criterion (BIC, also known as Schwartz Information Criterion):

$$BIC = -2 \log(L) - (N - k - 1) \log(N), \text{ where;}$$

L is the maximal likelihood obtained when estimating the β s;

k is the number of parameters of the model: 1 (β_b) for the Eystrup-specific models, and 2 (β_w and β_b) for the Paderborn-specific models;

N is the number of observations in the dataset (the number of non-infected animals) (Dohoo et al. 2003).

This criterion measures how closely the model fits the data (through the likelihood term L) and includes a penalty term that depends both on the number of parameters and on the size of the dataset. The smaller the value of the BIC, the better the model is.

All the numerical analyses were conducted using R (R Development Core Team 2006).

3. Results

Most of the animals infected with the Eystrup strain (experiment A) died or had to be killed for welfare reasons, and a single (non-infected) animal survived (Table I). Except for this latter pig, the maximal observed CS was always ≥ 15 (Figure 1). In experimentally inoculated animals, the average viraemia start day and the average clinical signs start day were identical (4 days PI). Viraemia and clinical signs started about 10 days later in contact infected animals (Table I).

No significant differences were observed between experimentally inoculated animals and contact infected pigs for the duration of viraemia, CS or for the average total CSs either in experiment A, nor in experiments B and C (Table I, Student's t-test: $p > 0.05$).

As expected, the Paderborn strain provoked a less marked mortality than the Eystrup strain (Table I). Maximal CSs were also lower: in experiment B, two animals showed a maximal CS ≥ 15 : an experimentally inoculated animal and a contact-infected pig. Both were killed afterwards for welfare reasons. In experiment C, CSs remained always ≤ 8 . Figure 1 also shows that in this experiment, after a few days with a CS of 0, some experimentally inoculated animals showed low CSs again. The corresponding symptoms were always very

slight and indefinite, such as a slightly reduced liveliness or a body shape indicating an empty stomach. However, at the end of the experiment, CSs were always zero and data censoring (that could bias R0 estimation) was considered negligible.

Total CSs were significantly higher with the Eystруп strain than with the Paderborn strain (Student's t-test: $p=0.02$), but no significant effect of strain on CS duration was observed (Student's t-test: $p>0.05$). Viraemia duration was significantly lower with the Eystруп strain than with the Paderborn strain (Student's t-test, $p=0.01$).

The duration of viraemia was comparable in experiments B and C (Student's t-test: $p>0.05$). Conversely, the duration of the clinical signs and the total CS were significantly higher in experiment B than in experiment C (Student's t-test, $p=0.04$ and $p=0.002$, respectively).

Following experimental inoculation with the Paderborn strain, average viraemia start date was 5 days PI in experiment B and 2 days PI in experiment C. In contact-infected animals, average viraemia start date was 19 days PI when contact was indirect (experiment B), and 13 days PI when contact was direct (experiment C) (Table I). The delay between average viraemia start date in experimentally inoculated animals and average viraemia start date in contact-infected pigs was thus 3 days longer with an indirect contact (experiment B: 14 days) than with a direct contact (experiment C: 11 days).

Comparable results were observed for clinical signs. In experimentally inoculated animals, average clinical signs start date was 8 days PI in both experiments. In contact-infected animals, average clinical signs start date was 25 days PI when contact was indirect (experiment B), and 21 days PI when contact was direct (experiment C) (Table I). The delay between average clinical signs start date in experimentally inoculated animals and average clinical signs start date in contact-infected pigs was thus 4 days longer with an indirect contact (experiment B: 17 days) than with a direct contact (experiment C: 13 days).

For the Eystrup strain, the estimated duration of the latent period was 4 days both for the viraemia and for the clinical signs datasets. For the Paderborn strain, these estimates were 4 days for viraemia and 8 days for clinical signs. The duration of the latent periods were used to compute, for each contact-infected animal, an infection date. Figure 2 compares this reconstructed incidence of infections (number of animals contaminated per day) with the infectivity released, for each of the three experiments and for each of the three datasets.

In the dataset based on viraemia (Figure 2, upper row), all of the contact animals were infected several days after all the experimentally infected animals had become viraemic. Contact animals were thus infected several days after the infectivity released by experimentally-inoculated animals had reached its maximal value. In experiments A and C, all of the contact animals were infected before the earliest viraemia in the contact group, demonstrating that all the contact animals were infected by the experimentally inoculated pigs. In experiment B (indirect contact), a single contact animal (number 6909) was infected at D20, four days after the beginning of viraemia in two other contact infected pigs. This animal may thus have been infected either by experimentally inoculated pigs in the neighbouring pen, or by the contact infected pig in its own pen.

With the qualitative clinical-based dataset (Figure 2, middle row) when the contact between experimentally inoculated and contact animals was indirect (experiments A and B), contact animals were infected several days after the appearance of clinical signs in experimentally inoculated animals. When the contact was direct (experiment C), the first infections occurred earlier, as they coincided with the beginning of the clinical signs in experimentally inoculated animals.

For the quantitative dataset based on clinical signs (Figure 2, bottom row), the relationship between the peak in clinical signs in experimentally inoculated animals and infection of contact animals appeared more variable.

The average total infectivity ($1/\alpha$) estimated from the datasets based on viraemia and that based on qualitative clinical signs were close, with higher values obtained for the Paderborn strain (11-12 days) than for the Eystrup strain (8-9 days) (Table II). Conversely, for the dataset based on quantitative clinical signs, the value obtained for the Eystrup strain was twice as high as the value obtained for the Paderborn strain.

With the qualitative clinical signs-based dataset, Paderborn and Eystrup strains gave similar between-pen contact rates (respectively 0.14 and 0.15). This was also the case for the quantitative clinical signs-based dataset (respectively 0.04 and 0.02). Differences between the strains were more marked for the viraemia-based dataset (Table II).

Whatever the dataset, between-pen R_0 estimates obtained for the Eystrup strain were close and ranged from 1.1 for the viraemia-based dataset, to 1.8 for the quantitative clinical-based dataset. An intermediate value was obtained for the qualitative clinical-based dataset (Table II). None of the Eystrup-specific R_{0b} were significantly >1 . The value of the Bayes Information Criterion (BIC) obtained for the quantitative clinical-based dataset was the lowest, followed by the qualitative clinical-based dataset and finally by the viraemia-based dataset (Table II).

The three datasets yielded similar values for the Paderborn-specific R_{0b} . These estimates were also close to those obtained for the Eystrup strain, and ranged from 1.0 (viraemia-based dataset) to 1.7 (quantitative clinical-based dataset). Again, these values were not significantly >1 .

Differences between datasets were observed for the Paderborn-specific within-pen R_0 : estimated R_{0w} ranged from 4.0 for the viraemia-based dataset to 12.2 for the quantitative clinical-based dataset. The value obtained from the qualitative clinical-based dataset was again intermediate (Table II). Whatever the dataset, this Paderborn-specific R_{0w} was significantly greater than 1 (lower bound of the 95% confidence interval >1). The lowest BIC

was obtained for the qualitative clinical-based dataset, followed by the quantitative clinical-based dataset and the viraemia-based dataset again had the highest BIC.

4. Discussion

Three CSFV transmission experiments conducted with two different strains (Eystrup and Paderborn) are reported here. The data obtained allowed us to study the effect of using different infectivity markers for transmission parameters estimation. Three different markers for individual infectivity were considered: viraemia data and clinical scores treated either qualitatively (presence/absence of clinical signs) or quantitatively. The three corresponding datasets allowed us to calculate R_0 estimates for the Eystrup and for the Paderborn strains. The corresponding models were ranked using the value an information criterion (BIC), to evaluate the pertinence of the individual infectivity markers studied.

For experiments with both strains, the viraemia-based dataset always had the highest BIC value, and was thus the worst model. According to the BIC, the best model was, for the Eystrup strain, the quantitative clinical-based dataset and, for the Paderborn strain, the qualitative clinical-based dataset. However, for each strain, the BIC differences between this best model and the next one were not very large. According to Raftery (1996), evidence for superiority of the model with the lowest BIC is weak when absolute difference is <2 , positive when absolute differences is ≥ 2 and <6 , and strong when the absolute difference is ≥ 6 . Following this interpretation, the BIC difference obtained for the Eystrup strain (6.4) would thus indicate strong evidence for superiority of the quantitative clinical-based dataset against the two others, which were similar. Similarly, the BIC difference obtained for the Paderborn strain (3) indicates the qualitative clinical-based dataset was superior to the two others, which were again similar to each other. The better fit of the clinical-based models suggests the existence of a relation between severity of the CSV symptoms and efficacy of CSFV

transmission. In infected herds, the most severely affected animals could thus play a prominent role in epizootic epidemic dynamic.

Furthermore, with the clinical-based datasets, estimated between-pen contact rates (β_b) appeared relatively independent of the strain as, for each dataset, the Eystруп-specific and the Paderborn-specific estimates gave similar values. This was not the case for the viraemia-based dataset. This suggests that, with the clinical-based datasets, the model allows a separation between two kinds of disease transmission determinants: excretion determinants (strain- and individual-specific) would be captured by the infectivity parameter ($1/\alpha$), whereas infection determinants (contact structure between animals) would be captured by the contact rates (β). However, these results need to be confirmed by other transmission experiments with different settings and different strains or animal types.

The R_0 values obtained from the viraemia-based dataset using the Paderborn strain, can be compared with estimates obtained earlier under comparable conditions (i.e. using animals of comparable weights and a moderately virulent strain).

Laevens et al. (1999) conducted a transmission experiment in slaughter pigs, to study the within-pen and the between-pen transmission of a moderately virulent CSFV strain (isolate obtained from the 1st herd affected in the 1993-1994 Belgian epidemic). Using the martingale estimator, Laevens et al. (1999) first reported a R_{0w} of 13.7 in slaughter pigs, with a large confidence interval (standard error: 13.7, i.e. 95% CI: -13.2–40). Klinkenberg et al. (2002) later refined these estimates using a likelihood-based method to obtain a smaller confidence interval for R_{0w} (15.5, 95% CI: 6.2–38.7) and to compute R_{0b} : 3.4 (95% CI: 1.5–7.4). All of these estimates were obtained using measurements of viraemia.

Data reported here show less marked differences between the efficacy of indirect contact (experiment B) and the efficacy of direct contact (experiment C) for CSFV transmission. The delay between average viraemia start in experimentally infected animals

and in contact infected pigs was 11 days with direct contact (experiment C) and 14 days with indirect contact. Despite this relatively small difference (3 days), $R0_w$ estimate is significantly >1 whereas $R0_b$ is not. This result is explained by the fact that, in experiment B (indirect contact), one of the four contact pigs was infected after some of the animals located in the same pen had started their viraemia: this latter pig may thus have been infected through within-pen contagion. Nevertheless, the $R0_w$ and $R0_b$ we obtained from the viraemia-based dataset, Paderborn strain (respectively 4.0 and 1.0) were clearly lower than the previously published estimates. Confidence intervals are large and this difference may be attributed to stochastic variations. Differences between the strains may also be incriminated. Moreover, concerning $R0_b$, the Laevens et al. (1999) estimate uncovered two transmission modalities (airborne transmission and transmission by contaminated clothes), whereas in the present case, only airborne transmission was possible. This design difference could also partially explain the $R0_b$ estimates variations. Finally, it is interesting to note that the $R0_w$ values obtained from the clinical-based datasets (7.6 and 12.2 for the qualitative and quantitative datasets) are closer to prior estimates than the values obtained from the viraemia-based dataset.

In recent years, detailed research studies have been conducted to design and enhance $R0$ estimation methods while adapting them to the type of data produced by transmission experiments: accurate (as many biological indicators can be measured) and sparse (since, for large animals such as pigs, the number of studied individuals is always low). Such studies allowed us to calculate more accurate $R0$ s, with smaller confidence intervals. However, besides this methodological research, little attention has been paid to the nature of the data used to estimate $R0$.

Basic reproduction ratio aims to quantify virus transmission. In principle, its estimation could thus be based upon any direct or indirect infectivity marker. Previous studies

have used viraemia-based markers, and Dewulf et al. (2002) compared viraemia and PCR based R0s in vaccinated sows, obtaining markedly different estimates. We chose to compare the usual viraemia-based estimates with two clinical-based estimates, using a CSF-specific score system for symptoms severity. The basic hypothesis is that the link between the (non-measurable) infectivity and the clinical scores is as close as the link between infectivity and viraemia. A better goodness of fit suggests that the clinical-based approach represents a cost-effective alternative to the viraemia-based approach.

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Table I. Estimated CSFV viraemic periods and observed CSF clinical scores in three transmission experiments conducted with a highly (A) or a moderately (B and C) virulent strain, and with direct (C) or indirect (A and B) contacts between experimentally inoculated and contact animals.

Exp.	Strain	Pen	Animal	Death ^a	Viraemic period ^b			Clinical scores ^c			
					Start	End	Duration	Start	End	Duration	Total
A	Eystrup	1	6885	11	5	11	7	4	11	8	79
		1	6889	10	5	10	6	4	10	7	60
		1	6900	12	2	12	11	4	12	9	97
		1	6899	10	5	10	6	3	10	8	58
		1	6913	10	2	10	9	4	10	7	54
	Average				4	11	8	4	11	8	70
	Contact	2	6895	21	14	21	8	14	21	8	101
		2	6903		14	23	10	14	27	14	94
		2	6915	27	16	27	12	14	27	14	189
		2	6916	21	16	21	6	14	21	8	85
B	Paderborn	1	6884	30	5	23	19	6	30	16	140
		1	6887		5	15	11	9	34	20	65
		1	6898	21	5	21	17	14	21	8	77
		1	6902	14	5	14	10	6	14	9	45
		1	6907		5	15	11	5	38	34	119
	Average				5	18	14	8	27	17	89
	Contact	2	6894		16	29	14	25	36	12	50
		2	6886	37	16	32	17	25	37	13	148
		2	6906		19	29	11	26	36	11	39
		2	6909		24	29	6	26	36	11	38
C	Paderborn	1	7187	15	2	15	14	7	15	9	35
		1	7227	18	2	18	17	10	18	9	31
		2	7214		2	7	6	8	24	3	3
		2	7239		2	12	11	8	42	13	24
	Average				2	13	12	8	25	8	23
	Contact	1	7199		13	27	15	18	33	14	16
		1	7216		16	16	1	24	30	2	2
		1	7241	25	13	25	13	15	25	10	33
		2	7189		13	21	9	23	42	11	17
		2	7203		13	21	9	15	33	5	5
		2	7234		13	27	15	30	30	1	1
	Average				13	23	10	21	32	7	12

^a Animals killed for welfare reasons.

^b Linearly interpolated from results of CSFV isolation from blood samples taken at days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28 (experiment A); 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28, 31, 33, 35, 38, 40, 42 (experiment B); and 0, 3, 7, 9, 11, 14, 16, 18, 12, 23, 25, 27, 28, 31, 35, 38, 45 (experiment C).

^c Measured daily. Start, end: first and last day for which clinical score is >0. Duration: number of days for which clinical score is >0. Total: global sum of clinical scores.

Table II. Comparison of the basic reproduction ratios estimated from results of CSFV isolation from blood (viraemia-based dataset) or from clinical scores (qualitative and quantitative clinical-based datasets) observed in three transmission experiments conducted with a highly (Eystруп) or a moderately (Paderborn) virulent strain and with direct or indirect contacts between experimentally inoculated and contact animals.

Strain	Parameter		Viraemia-based dataset	Clinical-based dataset	
				Qualitative	Quantitative
Eystруп	Latent period		4	4	4
	Avg. total infectivity	$1/\alpha$	8.3 (6.6–10.1)	9.2 (7.1–11.4)	90.8 (59.4–122.2)
	Between-pen contact rate	β_b	0.13 (0.05–0.35)	0.15 (0.06–0.40)	0.02 (0.01–0.05)
	Between-pen R_0	R_{0b}	1.1 (0.4–2.9)	1.4 (0.5–3.7)	1.8 (0.7–4.8)
	Bayes Information Criterion	BIC	21.6	20.4	14.0
Paderborn	Latent period		4	8	8
	Avg. total infectivity	$1/\alpha$	11.9 (9.7–14.1)	11.1 (7.6–14.6)	46.7 (25.1–68.4)
	Within-pen contact rate	β_b	0.33 (0.16–0.71)	0.69 (0.31–1.53)	0.26 (0.12–0.58)
	Between-pen contact rate	β_w	0.08 (0.03–0.25)	0.14 (0.05–0.39)	0.04 (0.01–0.10)
	Within-pen R_0	R_{0w}	4.0 (1.9–8.4)	7.6 (3.4–17.0)	12.2 (5.5–27.3)
	Between-pen R_0	R_{0b}	1.0 (0.3–3.0)	1.6 (0.6–4.3)	1.7 (0.6–4.6)
	Bayes Information Criterion	BIC	49.1	44.6	47.6

Bracketed: 95% confidence intervals.

Figure 1. Individual clinical scores (lines) and results of CSFV isolation from blood (filled circle: positive, empty circle: negative) in three transmission experiments conducted with a highly (A) or a moderately (B and C) virulent strain, and with direct (C) or indirect (A and B) contacts between experimentally inoculated and contact animals.

Clinical scores: measured daily. CSFV isolation from blood: samples taken at days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28 (experiment A); 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, 28, 31, 33, 35, 38, 40, 42 (experiment B); and 0, 3, 7, 9, 11, 14, 16, 18, 12, 23, 25, 27, 28, 31, 35, 38, 45 (experiment C). Stars: animals killed for welfare reasons.

Figure 2. Reconstruction of the animals' infectivity (left axis, solid line: experimentally inoculated, dashed: contact-infected) and of the contact animals' infection (bars, right axis) based upon results of CSFV isolation from blood (viraemia-based dataset, top) or upon clinical scores (clinical-based dataset, middle: qualitative, bottom: quantitative) observed in three transmission experiments conducted with a highly (A) or a moderately (B and C) virulent strain, and with direct (C) or indirect (A and B) contacts between experimentally inoculated and contact animals.

Infection of contact animals is assumed to occur 4 days before the 1st CSFV isolation from blood (top) or the 1st non-zero clinical score (middle, bottom, A), and 8 days before the 1st non-zero clinical score (middle, bottom, B, C)

Figure 1

Figure 2