

Modelling the time-dependent transmission rate for porcine circovirus type 2 (PCV2) in pigs using data from serial transmission experiments

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Six successive transmission trials were carried out from 4 to 39 days post inoculation (DPI) to determine the features of the infectious period for PCV2-infected pigs. The infectiousness of inoculated pigs, assessed from the frequency of occurrence of infected pigs in susceptible groups in each contact trial, increased from 4 to 18 DPI (0, 7 and 8 infected pigs at 4, 11 and 18 DPI, respectively) and then decreased slowly until 39 days post infection (4, 2 and 1 pigs infected at 25, 32 and 39 DPI, respectively). The estimated time-dependent infectiousness was fitted to three unimodal function shapes (gamma, Weibull and lognormal) for comparison. The absence of infected pigs at 4 DPI revealed a latency period between 4 and 10 DPI. A sensitivity analysis was performed to test whether the parametric shape of the transmission function influenced the estimations. The estimated time-dependent transmission rate was implemented in a deterministic SEIR model and validated by comparing the model prediction with external data. The lognormal-like function shape evidenced the best quality of fit, leading to a latency period of 8 days, an estimated basic reproduction ratio of 5.9 [1.8,10.1] and a mean disease generation time of 18.4 days [18.2, 18.5].

Keywords: modelling; transmission rate; porcine circovirus type 2; pigs

1. INTRODUCTION

The transmission rate parameter, defined as the mean number of new infections produced by a typical infectious individual per unit of time, is a pivotal parameter in epidemic models (Anderson & May 1991; De Jong 1995; McCallum *et al.* 2001). However, the estimation of this parameter in field conditions can provide a puzzling challenge as, in many infectious diseases, only the onset of clinical symptoms or the final outcome of the disease can be observed, the accurate time of occurrence of infection being unknown. Data from seroprevalence surveys can be used to a certain extent to estimate the force of infection by back-calculation methods (Sutton *et al.* 2006; Satou & Nishiura 2007).

Some transmission experiments have been carried out to estimate within- and between-pen transmission parameters for some pig diseases, e.g. classical swine fever (Klinkenberg *et al.* 2002) and foot-and-mouth disease (Eblé *et al.* 2006). These estimates were obtained

by a maximum likelihood method, taking into account the observed number of susceptible, infectious and recovered animals during the time course of the experiment based on the classic SEIR model (Kermack & McKendrick 1927). A basic assumption made in this compartmental model is that the infectious period is exponentially distributed. However mathematically convenient this assumption may be, it remains quite unrealistic from a biological point of view due to the considerable scatter of the infectious periods in relation to the mean duration of infection (Lloyd 2001). Another modelling approach, also known as the Kermack–McKendrick model, relies on models of time since infection in which the transmission rate is expressed individually in terms of the time elapsed since the occurrence of infection (Fraser *et al.* 2004; Fraser 2007). This kind of model is particularly used to model human immunodeficiency virus transmission as infectiousness has been shown to be higher for a few months post infection than during the asymptomatic period preceding AIDS development (Blythe & Anderson 1988; Pinkerton & Abramson 1996; Wawer *et al.* 2005). Time-dependent infectiousness reflects the

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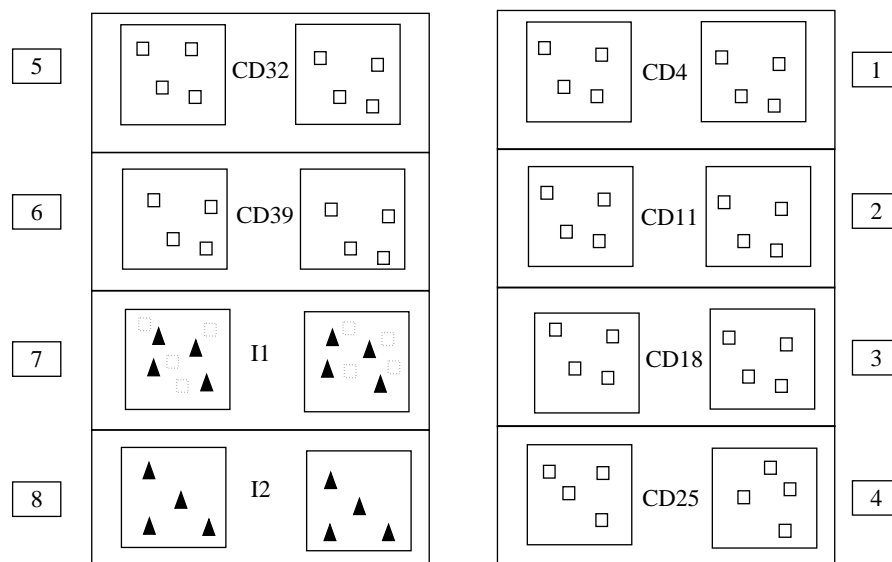


Figure 1. Experimental design of transmission experiment. Triangles, infected animals; squares, sentinel animals.

evolution of viraemia with time or, more precisely, the shedding pattern of the virus. In most cases, it is represented by a unimodal curve reflecting pathogen growth followed by an immune response (Fraser 2007).

Porcine circovirus type 2 (PCV2) is a small single-stranded DNA virus known to be the aetiological agent of post-weaning multisystemic wasting syndrome (PMWS) in pigs (Allan & Ellis 2000; Segalés & Domingo 2002). This disease is of great economic importance in the majority of pig-producing countries throughout the world owing to the increased mortality in severely affected farms and growth retardation in the case of subclinical infection (Madec *et al.* 2000; Segalés & Domingo 2002). Little is known about PCV2-specific infectiousness in growing pigs. A delay between inoculation of pigs with PCV2 and detection of the genome has been reported (Ladekjaer-Mikkelsen *et al.* 2002; Pensaert *et al.* 2004; Yu *et al.* 2007). These studies showed that the genome load became detectable at approximately 4 days post inoculation (DPI), increased rapidly up to 14 days and then decreased progressively. Many descriptive studies have been carried out to estimate the duration of viraemia or shedding of PCV2 by different routes (nasal, faecal, etc) from either field or experimental data (Calsamiglia *et al.* 2002; Rodriguez-Arrijoja *et al.* 2002; Lopez-Soria *et al.* 2005; Segalés *et al.* 2005; McIntosh *et al.* 2006). However, the demonstration of evidence for PCV2 particles was often based on polymerase chain reaction (PCR) or real-time PCR owing to the technical convenience and the impracticability of titrating virus from many samples with several repetitions over a relatively long period. The presence of a significant PCV2 genome load in sera or in nasal or rectal swabs cannot be considered as the evidence of infective viral particles because copies of DNA can be recovered in the absence of infective virions (McIntosh *et al.* 2006). The only evidence of infectiousness in a typical infected pig is its ability to infect a susceptible pig after a period of contact.

The aim of the present work was to assess the dynamics of infectiousness in inoculated pigs in terms of time elapsed since inoculation, under experimental

conditions. Six successive experimental transmission trials were carried out to estimate the number of infected animals in the contact groups for each contact period. Inoculated pigs were characterized in terms of their infectiousness as a function of time since infection (Fraser *et al.* 2004). Other epidemiological parameters such as the basic reproduction ratio (mean number of secondary cases produced by an infectious individual in a large susceptible population during its entire infectious period) and the mean disease generation time (mean time for a newly infected individual to infect a susceptible one) (Anderson & May 1991; Anderson *et al.* 2004; Svensonn 2007) were derived from the transmission rate function. Such parameters could be used to assess the effect of control strategies (e.g. vaccination, modifications in husbandry practices) on the course of infection. The estimated transmission rate was then incorporated into a deterministic SEIR model which took into account the time since infection, and the predictions of the model, in terms of incidence and prevalence, were compared with the results of a previous independent transmission trial.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Seventy-two specific pathogen-free (SPF), three-week-old weaned pigs, derived from the AFSSA (Agence Française de Sécurité Sanitaire des Aliments) SPF herd (Cariolet *et al.* 1994) and individually identified by an ear tag, were used. Eight pigs were kept as negative controls in room 0 and the 64 remaining pigs were randomly assigned to eight other groups housed in eight separate rooms in the air-filtered level-3 biosecurity facilities, each room containing two pens of four pigs (figure 1). Pigs in room 8 (group I2) were infected with PCV2 4 days after entering the facilities and kept as infected controls. Pigs in room 7 (I1) were infected at the same time and were mingled successively with susceptible pigs from rooms 1 to 6. The six groups of eight SPF pigs (CD4, CD11, CD18, CD25, CD32 and

CD39) were mingled, respectively, with the seeder group (I1) at 4, 11, 18, 25, 32 and 39 days post infection. Once mingled, they remained for 2 days with the seeders and were then returned to their original room to be monitored for PCV2 infection. Pigs were housed on flat decks (with a slatted floor 1 metre above the floor of the facility). The floor of the flat deck and of the facilities was cleaned thoroughly every day and just before receiving the contact pigs in order to prevent environmental contamination. The experiment was performed in accordance with EU and French regulations on animal welfare in experimentation.

2.2. Inoculum

Infected pigs were inoculated at day 0 with 6 ml (5 ml intratracheal +1 ml intramuscular) of a PCV2 suspension (10^5 TCID₅₀ ml⁻¹) derived from pooled tracheobronchial lymph nodes of a PMWS-affected pig obtained in a previous experiment (Grasland *et al.* 2005).

2.3. Observation and sampling

Pigs were examined daily for clinical signs (rectal temperature, cough, sneezing, individual weight, feed consumption) until they were killed at the end of the experiment in the case of inoculated seeder and control pigs (I1 and I2), or at least 35 days post mingling in the case of contact pigs. Blood samples were taken weekly for PCV2 serology and real-time PCR. Euthanasia was carried out by anaesthesia (Nesdonal; Merial, Lyon, France) followed by exsanguinations. All pigs were necropsied and their organs examined.

2.4. Serology

The PCV2 antibodies in the weekly collected sera were detected using an ELISA test based on the recognition of a recombinant PCV2 capsid protein/GST fused protein and a GST protein (Blanchard *et al.* 2003). Those samples with an OD ratio above 1.5 were considered positive for PCV2 antibodies (sensitivity: 0.98 and specificity: 0.95, taking IPMA (immunoperoxidase monolayer assay) as a reference).

2.5. Quantification of PCV2 genomes by real-time polymerase chain reaction

DNA was extracted from 200 µl of each tested serum using the Wizard SV96 genomic DNA purification system (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. Elution was performed with 250 µl of sterile H₂O, and 5 µl of this extract, corresponding to 4 µl of serum, was used as a template for PCV2 TaqMan PCR. Controls were carried out during DNA extraction by replacing serum with phosphate-buffered saline (PBS), for every five samples in order to check for any PCV2 contamination.

The number of PCV2 genome copies was assessed by a real-time PCR based on TaqMan technology (Blanchard *et al.* 2004). Briefly, the designed PCV2-

specific primers 5'-GGGAGCAGGGCCAGAATT-3' (410–427) and 5'-CGCTCTGTGCCCTTTGAA-TACT-3' (473–452) target the PCV2 ORF2 region (GenBank accession no. AF201311) and allow the amplification of a 64 bp fragment. The TaqMan probe 5'-ACCTTAACCTTTCTTATTCTG-3' (430–450) was labelled with the fluorescent reporter dye FAM (6-carboxyfluorescein) at the 5' end and with the non-fluorescent quencher (NFQ) associated with the minor groove binder at the 3' end. A DNA solution of a plasmid carrying a single copy of the PCV2 genome was serially diluted and used to generate a standard curve of quantification. The reactions were performed on an ABI Prism 7000 thermocycler (Applied Biosystems, Foster City, CA, USA).

2.6. Estimation of the transmission parameter related to time since inoculation ($\beta(\tau)$)

Let τ and $\beta(\tau)$ be the time since inoculation and infectious potential of inoculated animals, respectively. $\beta(\tau)$ is the mean number of animals that could be infected by an inoculated one at time τ after inoculation. Hence, the number of individuals potentially infected by a single infectious pig over a time interval of $[t_0, t_1]$ is given by $\int_{t_0}^{t_1} \beta(\tau) d\tau$. Thus, the basic reproduction ratio R_0 , which is equal to the average number of infected individuals produced by a single infectious one during its entire period of infectiousness, can be computed by integrating $\beta(\tau)$ over the entire infectious period, or equivalently over the period for which $\beta(\tau)$ is strictly positive. The probability of one pig escaping infection during a 2 day contact period (between t_i and t_{i+1}) is given by $q_i = \exp(-I \int_{t_i}^{t_{i+1}} \beta(\tau) d\tau)$, I being the number of infectious animals and t_i and t_{i+1} corresponding to the first and last contact days of contact group CD_{*i*}. The number of new infections during this interval follows a binomial distribution with parameters $S=8$ (number of susceptible individuals at each contact trial) and $p_i=1-q_i$. The log likelihood of this binomial distribution is given by the expression $\sum_{i=1}^6 \log \binom{S}{C_i} + \sum_{i=1}^6 (C_i \log(p_i) + (S-C_i) \log(q_i))$, where C_i is the number of cases in each contact trial. The infectious potential $\beta(\tau)$ is estimated by maximizing this function. The integrals were computed using the *quad* (quadratic approximations of integrals) function in MATLAB (Matlab 1984–2007 The MathWorks, Inc.) software and the log likelihood was maximized by using the 'quasi-Newton line search' algorithm displayed by the *fminunc* function (unconstrained minimization of a multivariable function) in the MATLAB software. Confidence intervals for the parameter estimates of the $\beta(\tau)$ function were derived from the Hessian matrix of the parameters provided in MATLAB's *fminunc* function.

The mean disease generation time, which, by definition, is the mean time for a newly infected individual to infect a susceptible one (Anderson & May 1991; Anderson *et al.* 2004; Svensonn 2007), can also be computed, $T_g = \int_0^\infty (\tau \beta(\tau) / \int_0^\infty \beta(s) ds) d\tau$ (Fraser *et al.* 2004).

2.7. Epidemic model including the time-dependent transmission rate

The time-dependent transmission rate function was incorporated into a deterministic epidemic model in order to study the effect of the time-dependent infectiousness on the course of infection. The model was a closed SEIR model. However, the ‘Exposed’ class was not explicitly represented because the latency period was assumed to be constant, which implies that each newly infected individual was introduced in class E for a constant duration. This is represented by setting $\beta(\tau)$ at 0 for the estimated duration of the latency period. Thus, our model was reduced to an SIR model with time-dependent infectiousness. Animals were considered as infectious throughout their infectious period, i.e. until the time when infectiousness faded out. In this model, the transmission rate was considered to be negligible when it fell below 0.001, which corresponded to x days post infection [$\beta(x) < 0.001$]. Hence, the model is expressed by the integro-differential system

$$\frac{dS}{dt} = -\frac{S(t)}{N} \int_0^\infty \beta(\tau) I(t, \tau) d\tau, \quad (2.1)$$

$$\frac{\partial I}{\partial t} + \frac{\partial I}{\partial \tau} = -\delta_x(\tau) I(t, \tau), \quad (2.2)$$

$$\frac{dR}{dt} = \int_0^\infty \delta_x(\tau) I(t, \tau) d\tau, \quad (2.3)$$

where t is the time and τ the time since infection. N is the total number of pigs and δ_x is the Dirac measure corresponding to the fixed length of the infectious period (i.e. $\delta_x(\tau) = \infty$ if $\tau = x$, $\delta_x(\tau) = 0$ if $\tau \neq x$ and $\int_{-\infty}^{+\infty} \delta_x(\tau) d\tau = 1$). Moreover, for the boundary condition we have

$$I(t, 0) = \frac{S(t)}{N} \int_0^\infty \beta(\tau) I(t, \tau) d\tau \quad \text{if } t > 0. \quad (2.4)$$

The initial condition, $I(0, a)$, describes the distribution of infectives according to the time since infection, i.e. the initial number of infectious individuals infected a time units ago.

2.8. Validation with external data

The model outputs were compared with previous data derived from four experimental transmission trials (Andraud *et al.* 2008). In each of these trials, contacts were made between four inoculated pigs and four susceptible ones. The inoculations were performed as in the present experiment (same route and dose). All susceptible animals were found to be infected at the end of the trial. The first positive genome loads were detected on day 21 post contact in susceptible pigs and the numbers of infected pigs increased thereafter until day 28 post contact. The pigs were monitored twice a week, to obtain the cumulative incidence and prevalence between 2 sampling days.

The outputs of the model were compared with those external data by supposing that all initially infectious individuals were infected at time $t=0$. In this way, the conditions of an experimental transmission trial

starting with the inoculation of I_0 individuals at time $t=0$ were fulfilled. Mathematically, this assumption resulted in the following initial condition:

$$S(0) = S_0 \quad I(0, a) = I_0 \delta_0(a) \quad R(0) = 0, \quad (2.5)$$

where δ_0 is the Dirac measure, $S_0=4$ and $I_0=4$.

Quality of fit of predicted data with observed number of infections was assessed with a χ^2 goodness-of-fit test and the coefficient of determination (R^2) calculation.

3. RESULTS

3.1. Clinical and post-mortem findings

Very mild symptoms were observed during the experiment with only pyrexia (higher than 40.5°C) in inoculated pigs (2/8 in I1 and 3/8 in I2) 20 days post infection. No symptom was observed in contact groups, except pyrexia (higher than 40.5°C) in group CD18 (1/8) between 14 and 20 days post contact. No lesion was detected at necropsy except in two pigs from group I1 (moderate enlargement of kidneys with white foci). Histopathological investigations did not reveal any possibly PMWS-related lesions (non-specific infarction). Lymph nodes were normal with no specific PCV2-related lesions.

3.2. PCV2 genome load and serological results in inoculated pigs

The eight separately housed, negative control pigs remained PCV2 negative until the end of the experiment when the last group was slaughtered (data not shown).

The PCV2 genome load in serum increased from 0 to 11 days post infection in inoculated pigs, reaching a peak (10^6 genome copies per ml on average at 11 DPI) and then decreased steadily until the end of the experiment.

All the inoculated pigs were seropositive 18 days after inoculation and remained so until the end of the experiment. No discrepancy between inoculated control [I2] and inoculated seeder [I1] groups was observed for either PCV2 genome load or serological results (figure 2).

3.3. Infections in contact groups

Two types of infection in contact groups were defined, based on PCR and serological results: primary infections, which occurred during the 2 day contact with inoculated pigs, and secondary infections, which took place once the contact groups returned to their own room. An average delay of 15 days was observed between primary and secondary infections. Within a specific pen, the infections detected 15 days after the first infections could not be related to those contracted with inoculated pigs because

- (i) the genome load in inoculated pigs was homogeneous and
- (ii) the contact rate between inoculated and contact pigs could reasonably be assumed to be uniform.

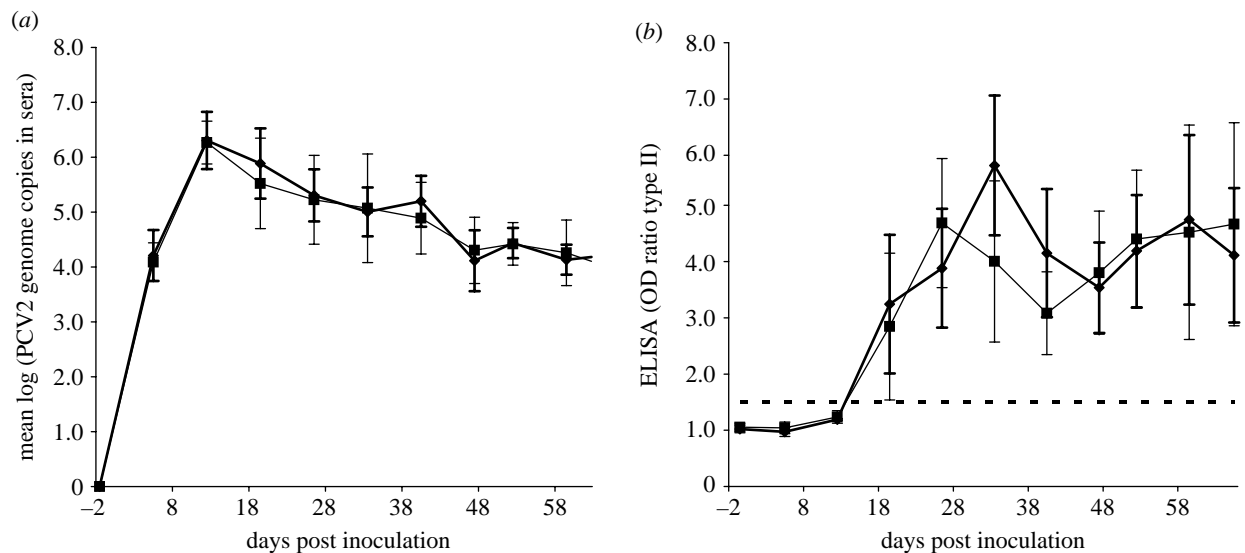


Figure 2. PCV2 genome load in sera and serological response of inoculated pigs for the control and contact groups. (a) PCV2 genome load and (b) serological response of inoculated pigs from the control (thin lines) and contact (thick lines) groups. ELISA positivity threshold (b): 1.5 (dashed line).

During this time interval of 15 days, the genome load of primary infected pigs increased and primary infected pigs were able to infect the susceptible pigs left in their room.

Contact pigs from group CD4 did not show any seroconversion until slaughtering (35 days post contact) and did not show PCV2 genome in sera throughout the monitoring period, showing that the inoculated pigs were not yet infectious at 5 DPI. All pigs from groups CD11 and CD18 seroconverted after the 22nd day post contact, but one pig in group CD11 was identified as a secondary infection owing to late seroconversion and delayed increase of the PCV2 genome load (15 days after detection of the first infected animal). The number of primary infected pigs then fell to 4, 2 and 1 pig in groups CD25, CD32 and CD39, respectively (figure 3). Secondary infected pigs (15 days or more of interval between the first infected pigs and these secondary infected animals) were also identified, on the basis of both PCR and ELISA data, in these three groups.

3.4. Parameter estimation

Assumptions were made as to the shape of the most probable infectious curve according to the number of pigs infected within each contact group. The curve was deemed unimodal, with the mode between 11 and 18 DPI. Moreover, the function was deemed to be left skewed, lower bounded, owing to the latent period, and upper bounded since only one of the eight susceptible pigs in group CD39 had been infected, the upper bound being more than 39 days. According to these assumptions, three function shapes were tested,

(i) a gamma-like function defined by

$$\beta(\tau) = \begin{cases} R_0 f_\gamma(\tau - \text{Lat}, k, \theta) & \text{if } \tau \geq \text{Lat}, \\ 0 & \text{if } \tau < \text{Lat}, \end{cases}$$

(ii) a Weibull-like function

$$\beta(\tau) = \begin{cases} R_0 f_W(\tau - \text{Lat}, k, \theta) & \text{if } \tau \geq \text{Lat}, \\ 0 & \text{if } \tau < \text{Lat}, \end{cases}$$

(iii) a lognormal-like function and

$$\beta(\tau) = \begin{cases} R_0 f_{\text{Ln}}(\tau - \text{Lat}, k, \theta) & \text{if } \tau \geq \text{Lat}, \\ 0 & \text{if } \tau < \text{Lat}, \end{cases}$$

where f_γ , f_W and f_{Ln} are the gamma, Weibull and lognormal probability density functions, respectively, with parameters k and θ , and with Lat, the duration of latency. k and θ are, respectively, the shape and scale parameters of the gamma and Weibull distributions and the mean and variance of the lognormal distribution. Thus $\beta(t)$'s characterization requires the estimation of three parameters, k , θ and R_0 .

Since no pig in group CD4 (which were placed in contact on days 4 and 5 post inoculation) became infected and almost all pigs in the next contact group were infected, the duration of latency was considered to range between 6 and 10 days post infection. Table 1 summarizes all the parameter estimates for the latency duration from 6 to 10 days post infection. After the implementation of the time-dependent epidemic model, a sensitivity analysis was carried out to determine the most accurate shape for $\beta(t)$. Only three curves were found to be the most consistent with both observed infections during serial contacts (figure 4) and the external data (cumulated incidence between 2 sampling days, figure 5a)

- (i) the gamma-like function with an 8 day duration of latency,
- (ii) the Weibull-like function with a 9 day duration of latency, and
- (iii) the lognormal-like function with an 8 day latency.

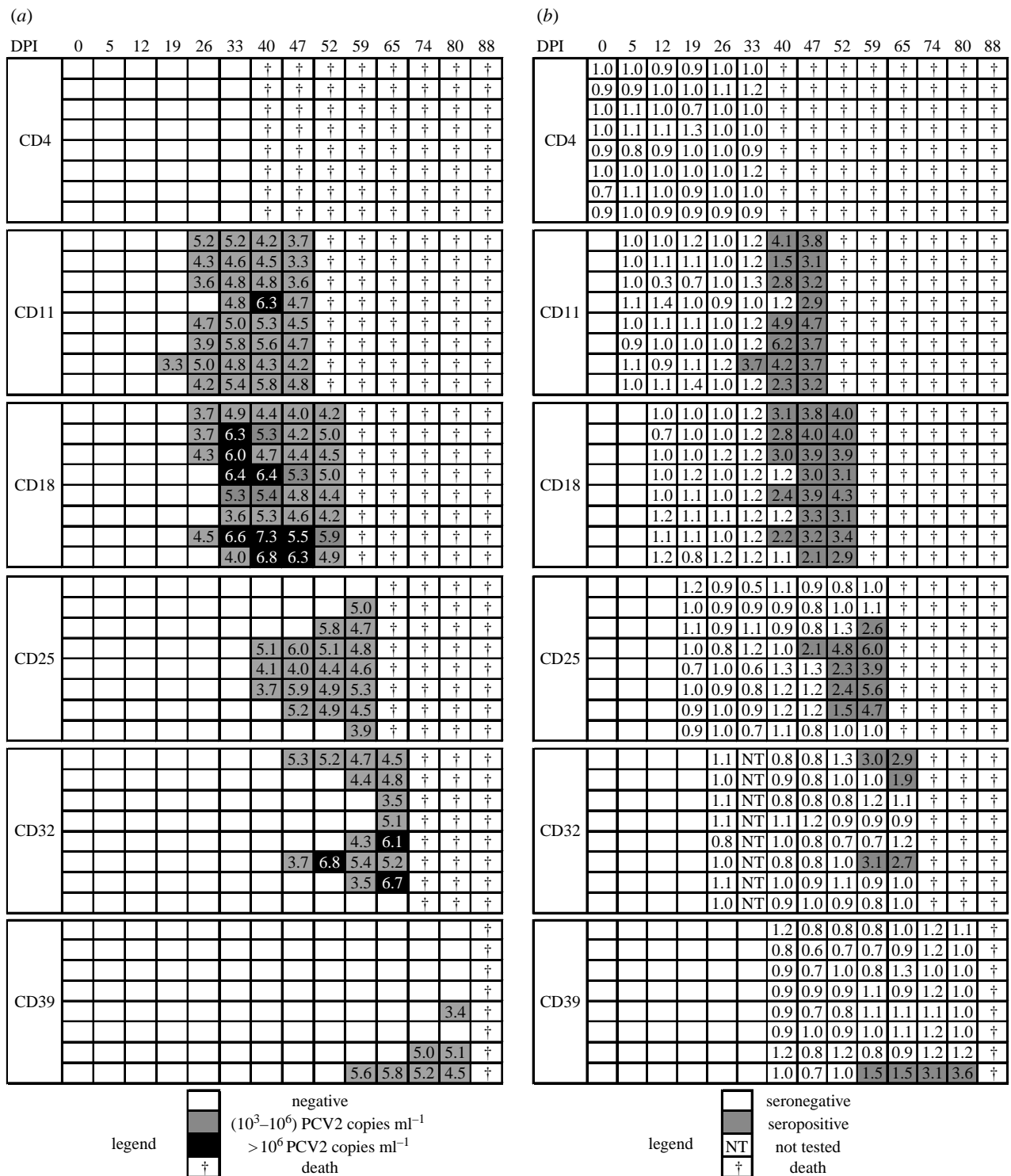


Figure 3. Kinetics of infection in contact pigs. (a) Individual genome loads in sera and (b) individual serology of contact pigs.

However, the best results were obtained with the lognormal function with a corresponding latency period of 8 days. Whatever the shape of the parametric function, the predicted cumulated incidence and prevalence based on the model with a time-dependent transmission rate showed a better overlap with the observed data than those predicted by a classical SEIR model with the corresponding parameters (figure 5).

The graph of the infectiousness function $\beta(t)$ (lognormal shape) is provided in figure 6. The mode of this function was situated at 15 DPI, which

corresponds both to the period of genome load increase and seroconversion in inoculated pigs. $\beta(t)$ was found to be <0.001 beyond 55 days post infection.

Parameter estimates and confidence intervals are summarized in table 1. The basic reproduction ratio (R_0) for PCV2 infection was estimated by integrating $\beta(t)$ over the entire infectious period: $R_0 = \int_0^\infty \beta(\tau) d\tau = 5.9$ [1.8,10.1]. The mean disease generation time (mean time for a newly infected animal to infect a susceptible one) can also be computed, $T_g = \int_0^\infty (\tau\beta(\tau) / \int_0^\infty \beta(s) ds) d\tau = 18.4$ days [18.2,18.5].

Table 1. Sensitivity analysis on the parametric shape of the function representing the time-dependent transmission rate: estimates and 95% confidence intervals (CIs) of the parameters of the infectious potential function distribution, estimated basic reproduction ratio R_0 , mean generation time T_g and goodness of fit tests on observed and external (Andraud *et al.* 2008) data according to the assumed duration of the latent period. ($\beta(\tau)$, parametric function of infectious potential; k, θ , parameters of $\beta(\tau)$; R_0 , basic reproduction ratio defined as the average number of secondary infections occurring from a single infected animal during its infectious period in a totally susceptible population; T_g , mean generation time defined as the sum of the mean latent period and the mean infectious period; R^2 , coefficient of determination; ND, not defined.)

| function shape | latent period (days) | parameters (CI) | | | | goodness of fit on observed data | | | goodness of fit on external data | | |
|----------------|----------------------|------------------|------------------|----------------|---------------------------------|----------------------------------|------------|-------|----------------------------------|------------|-------|
| | | k | θ | R_0 | mean generation time T_g (CI) | χ^2 | p -value | R^2 | χ^2 | p -value | R^2 |
| gamma | 6 | 3.6 (0.7,6.6) | 3.4 (1.1,5.7) | 5.3 (3.3,7.2) | 18.3 (6.7,25.4) | 0.31 | 0.98 | 0.96 | 7.67 | 0.36 | 0.23 |
| | 7 | 3.2 (1.2,5.3) | 3.5 (1.2,5.9) | 5.2 (3.2,7.2) | 18.4 (8.3,27.4) | 0.3 | 0.98 | 0.97 | 4.76 | 0.69 | 0.5 |
| | 8 | 2.9 (1.5,4.2) | 3.7 (1.3,6.1) | 5.2 (3.1,7.4) | 18.6 (9.9,27.5) | 0.3 | 0.99 | 0.97 | 2.56 | 0.86 | 0.9 |
| | 9 | 2.5 (1.6,3.3) | 3.9 (1.4,6.4) | 5.2 (2.9,7.6) | 18.7 (11.3,26.5) | 0.29 | 0.99 | 0.97 | 2.91 | 0.82 | 0.86 |
| | 10 | 2.1 (1.7,2.6) | 4.1 (1.6,6.7) | 5.3 (2.5,8.1) | 18.7 (12.7,24.9) | 0.28 | 0.99 | 0.97 | 7.44 | 0.28 | 0.53 |
| Weibull | 6 | 12.9 (8.5,17.4) | 1.7 (1.5,1.9) | 5.4 (3.3,7.5) | 17.5 (13.6,21.2) | 0.31 | 0.99 | 0.96 | 10.76 | 0.15 | 0.11 |
| | 7 | 12.2 (8.6,15.9) | 1.6 (1.5,1.8) | 5.2 (3.3,7.1) | 17.9 (14.7,20.9) | 0.31 | 0.98 | 0.95 | 7.55 | 0.37 | 0.23 |
| | 8 | 11.5 (8.5,14.4) | 1.6 (1.4,1.7) | 5.0 (3.3,6.8) | 18.3 (15.7,20.7) | 0.31 | 0.98 | 0.96 | 4.41 | 0.62 | 0.55 |
| | 9 | 10.7 (8.3,13.1) | 1.5 (1.4,1.6) | 4.9 (3.3,6.6) | 18.6 (16.6,20.6) | 0.3 | 0.99 | 0.96 | 2.84 | 0.83 | 0.93 |
| | 10 | 9.8 (7.9,11.8) | 1.4 (1.4,1.5) | 4.9 (3.2,6.5) | 18.9 (17.2,20.5) | 0.3 | 0.99 | 0.96 | 7.08 | 0.31 | 0.56 |
| lognormal | 6 | 2.42 (2.41,2.43) | 0.47 (0.46,0.48) | 5.6 (2.7,8.6) | 18.4 (18.3,18.6) | 0.23 | 0.99 | 0.98 | 5.89 | 0.55 | 0.33 |
| | 7 | 2.32 (2.31,2.32) | 0.50 (0.49,0.51) | 5.7 (2.3,9.2) | 18.4 (18.3,18.6) | 0.22 | 0.99 | 0.98 | 2.73 | 0.91 | 0.77 |
| | 8 | 2.20 (2.19,2.21) | 0.54 (0.53,0.56) | 5.9 (1.8,10.1) | 18.4 (18.2,18.5) | 0.2 | 0.99 | 0.98 | 1.15 | 0.98 | 0.99 |
| | 9 | 2.06 (2.05,2.07) | 0.60 (0.58,0.61) | 6.3 (0.7,11.9) | 18.2 (18,18.4) | 0.17 | 0.99 | 0.98 | 3.6 | 0.73 | 0.8 |
| | 10 | 1.86 (1.85,1.88) | 0.67 (0.66,0.69) | 7.0 (ND,16.08) | 17.9 (17.7,18) | 0.15 | 0.99 | 0.98 | 8.95 | 0.17 | 0.5 |

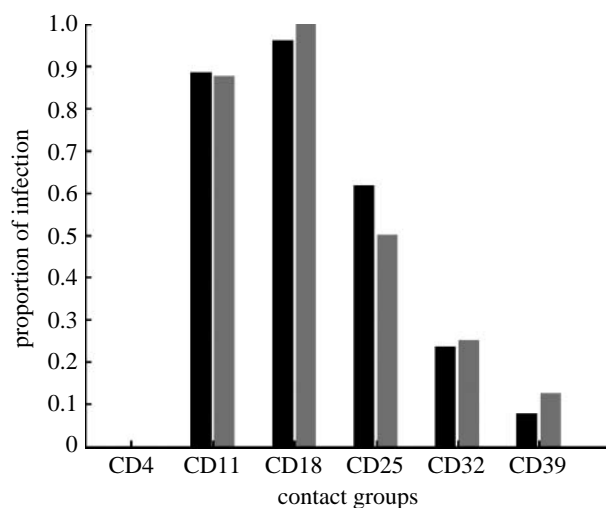


Figure 4. Proportion of observed (grey bars) and predicted (black bars) (time-dependent transmission rate model with lognormal-like function) infections according to contact groups. Each group was composed of eight susceptible pigs mingled with the inoculated pigs for a 2 day long contact at 4, 11, 18, 25, 32 and 39 DPI for groups CD4, CD11, CD18, CD25, CD32 and CD39, respectively.

4. DISCUSSION

PCV2 is now recognized as the main agent involved in PMWS. However, although many attempts have been made to reproduce the disease by inoculating conventional piglets (Allan *et al.* 1999; Kennedy *et al.* 2000), only mild lesions were reproduced by a sole PCV2 inoculation without immune enhancement (Krakowka *et al.* 2001; Grasland *et al.* 2005) or co-infection (Allan *et al.* 2000; Krakowka *et al.* 2000; Harms *et al.* 2001). The results of this study are consistent with these previous observations since no clinical signs and no specific lesions could be observed during the experiment with the sole PCV2 infection. All inoculated pigs were successfully infected and similar genome loads were attained in sera as reported in field conditions (Sibila *et al.* 2003). Moreover, virus transmission occurred from inoculated pigs to susceptible ones and no difference in genome loads could be observed between inoculated and contact pigs.

The pigs were inoculated as in a previous study on experimental reproduction of PMWS (Albina *et al.* 2001), i.e. by both intratracheal and intramuscular routes. Although these inoculations were quite ‘unrealistic’ as regards infection in the field, they produced a homogeneous and comparable population of infectious pigs for each successive contact trial. The use of secondary infected pigs as seeders to infect contact ones, as in another transmission experiment on *Actinobacillus pleuropneumoniae* (Velthuis *et al.* 2003), could have been relevant because it would have reproduced, in a more natural way, infections occurring within a farm. However, it would have been difficult to assess the infectious status and the genome loads of such infected seeders on the time scale of the experiment and this is of primary importance since all the infectious seeders were assumed to have homogeneous infectious potential throughout the entire trial.

Moreover, adding such supplementary phases would have required reducing the number of serial transmission trials owing to the need to maintain the required space per pig.

No difference in PCV2 genome load or serological response could be evidenced between inoculated control and inoculated contact pigs, showing that serial contacts did not enhance viral replication in the pigs subjected to serial mingling with sentinel pigs.

Six successive contact trials were carried out to determine transmission behaviour over time. This approach differs from classic transmission trial experiments (Klinkenberg *et al.* 2002; Eblé *et al.* 2006) since a new pool of susceptible animals is subjected to a 2 day contact each week. Each group, once returned to its original room, has its own dynamics. Hence the algorithms generally used in transmission experiments and based on an epidemic model applied to the closed population involved in the experiment could not be used in our case and a different approach was necessary.

An initial increase in the number of primary infected animals was observed followed by a slow decrease. Real-time PCR and serology indicated the presence of secondary infected animals, i.e. those infected by primary cases after they returned to their own room. In comparison with the primary cases, genome load increase and seroconversion were delayed in these animals. Hence these infections were not taken into account in the estimation of transmission rate since they did not involve the inoculated animals whose infectiousness was under investigation. Moreover, the estimated mean generation time of 18.4 days (18.2; 18.5) confirmed that the presumed secondary infections were actually due to primarily infected animals once they had returned to their room. Even in the last trial, one susceptible animal became infected. A supplementary trial 46 DPI probably would have indicated an absence of primary infections, but this experimental evidence is lacking and no definite conclusion can be drawn. However, both the observed decrease in the number of infections and the quality of fit of the predicted number of infections according to the chosen distributions for the transmission parameter confirmed the adequacy of the available data. The sensitivity analysis showed that the choice of the function shape for $\beta(\tau)$ did not largely influence the time when the transmission rate became negligible.

The increasing phase of transmission rate function between 8 and 15 days post infection can be linked to the increasing genome load in the serum as described in many studies on PCV2 (Ladekjaer-Mikkelsen *et al.* 2002; Pensaert *et al.* 2004; Yu *et al.* 2007). This suggests a strong relationship between genome load and transmission efficacy during the initial phase of an epidemic. However, PCV2 DNA can be found in tissues and serum for a long time (up to 70 days) after the occurrence of infection (Rodriguez-Arrijoja *et al.* 2002; Shibata *et al.* 2003; McIntosh *et al.* 2006). We found that the transmission rate decreased between 15 and 55 days post infection, became negligible thereafter, but that a relatively significant genome load persisted in inoculated animals. There could be two reasons for this apparent contradiction: (i) a significant PCV2 genome

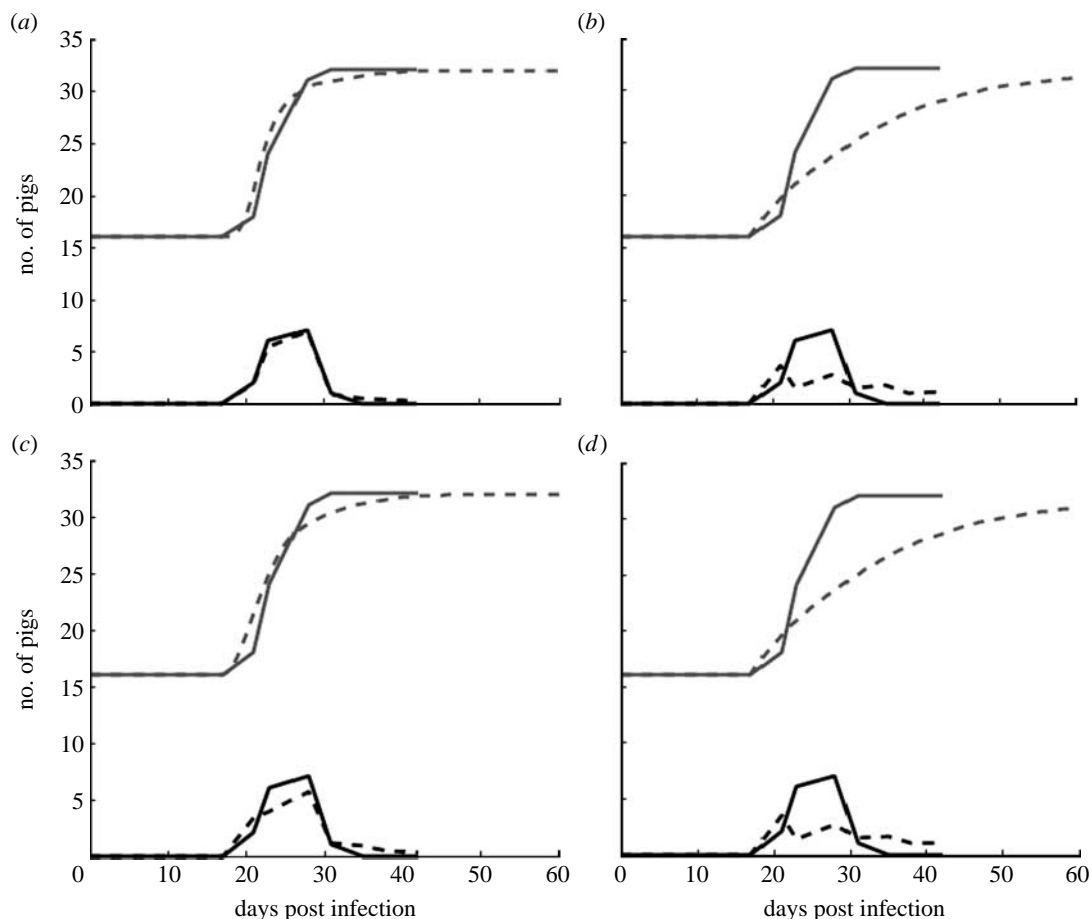


Figure 5. Comparison of model outputs with previous independent experimental transmission results (four repetitions of a 4*4 transmission trial). Observed incidence (black curve) and prevalence (grey curve) from previous independent experimental transmission trials compared with predicted incidence (dashed black curve) and prevalence (dashed grey curve) derived from (a,c) time-dependent model and (b,d) classical SEIR model outputs. (a) Time-dependent model with a lognormal-like shape for transmission rate function (parameters given in table 1). (b) Classic SEIR model with parameters corresponding to (a): fixed latent period of 8 days; transmission parameter: 0.13; average duration of infectious period: 47 days, leading to a basic reproduction ratio of 5.9 equivalent to the one computed in the lognormal-like time-dependent model. (c) Time-dependent model with a gamma-like shape for transmission rate function (parameters given in table 1). (d) Classic SEIR model with parameters corresponding to (c): fixed latent period of 8 days; transmission parameter: 0.12; average duration of infectious period: 42 days, leading to a basic reproduction ratio of 5.2 equivalent to the one computed in the gamma-like time-dependent model.

load in sera or in nasal or rectal swabs cannot be considered as the evidence of the presence of infective viral particles because copies of DNA can be recovered without infective virions (McIntosh *et al.* 2006) and (ii) the decline in transmission rate coincides with the published delay (between 14 and 21 DPI) in appearance of neutralizing antibodies against PCV2 (Meerts *et al.* 2006; Fort *et al.* 2007; Opriessnig *et al.* 2007) and the decline in genome load (Pensaert *et al.* 2004). This suggests a typical infectious process governed by viraemia and the development of an immune response as in many viral infections. Biologically, it has been demonstrated that the transmission rate is upper bounded, which means that even if viral DNA can be found in serum long after infection, this does not imply that the animals remain infectious for the same period. As a consequence, Q-PCR results should not be interpreted as a measure of infectiousness for PCV2. From a modelling point of view, the infectious status is defined by the ability of an individual to shed and transmit the virus to susceptible ones. According to

our findings from serial transmission experiments, this could not be addressed by virus monitoring (Q-PCR and serology).

The infectious potential of all inoculated pigs was assumed to be homogeneous, although slight differences in individual genome loads were observed. This individual variability was not taken into account since all pigs were inoculated with the same route and same dose at the beginning of the experiment. Moreover, pigs were raised in a rather unnatural but highly controlled environment (level-3 biosecurity facilities with air filtration, small pens with eight pigs per pen during contact period, SPF status) limiting the influence of individual variability on the infection dynamics. Even if the size of the population under study was small in the experimental setting, deterministic simulations were deemed reliable in this context of a strictly controlled experiment. In conventional pig farms, because naturally infected pigs are more likely to differ in their infectiousness, the application of these results should take into account this variability between individuals.

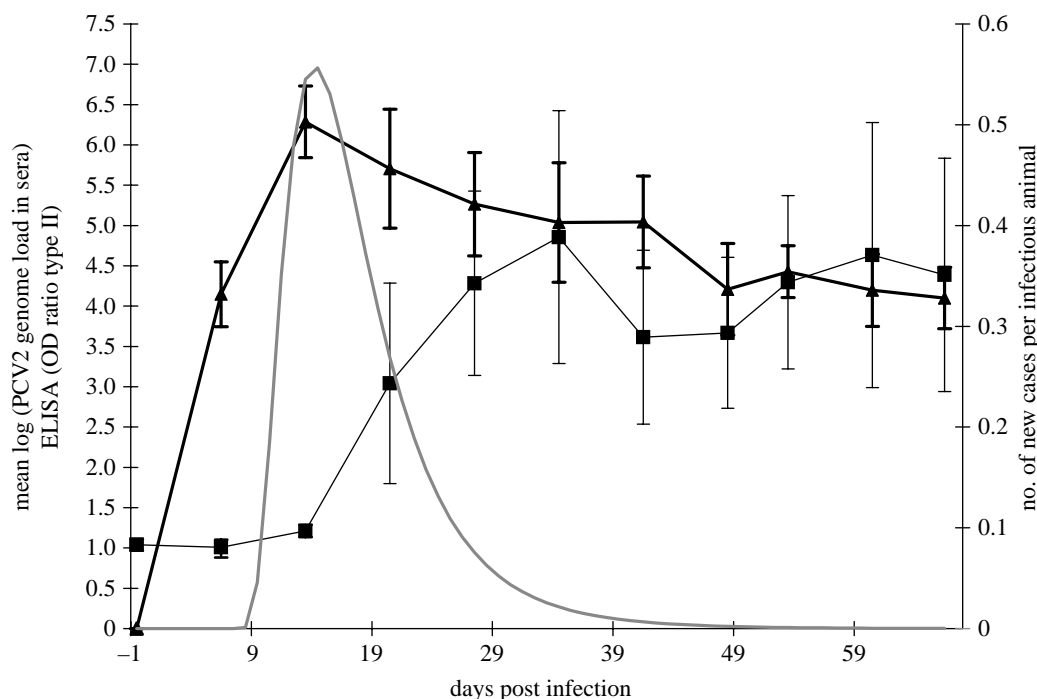


Figure 6. Relation between genome load, serology and the lognormal-like transmission rate function. Mean genome load (thick black curve) and serology titre (thin black curve) in relation to transmission rate function (full grey curve).

In field conditions, typical finishing facilities are divided into pens housing from 10 to 30 pigs. This non-homogeneous mixing leads to within- and between-pen transmission. Our results can be used to model within-pen dynamics. Between-pen transmission was quantified previously (Andraud *et al.* 2008) and this estimate could be used together with the shape of the transmission function obtained in the present study to model overall infectious process within a finishing room. The basic reproduction ratio, R_0 , is defined as the mean number of secondary infections occurring from a single infected animal during its infectious period in a large susceptible population. Hence, the R_0 estimates in this study are only theoretical parameters, indicating how the virus would spread if the population would be randomly mixed. Considering the real structure of a pig farm, the present results would need to be implemented within a more detailed and accurate representation of the population (variability between individuals, structure of contact).

The estimated time-dependent transmission rate was introduced into a time-dependent epidemic model to compare the outputs of our model with data from a previous transmission experiment. This model was able to reproduce the observed incidence and prevalence more consistently than a classical SEIR model with a constant transmission parameter. This shows that the dynamics of infection, in experimental conditions, could be fully explained by the estimated time-dependent transmission rate. In a classic SEIR model, individuals once they have become infectious are transferred to the 'recovered' compartment at a constant rate (modelling an exponentially distributed infectious period) and the transmission parameter is considered as constant. This leads to a decrease in the force of infection at the beginning of the infectious

process before the appearance of primary infected individuals. Comparisons with observed experimental data show that the infection process is more appropriately reproduced as a time-dependent model. From our results three function shapes were in adequacy with the observed data on infection dynamics, leading to the conclusion that the latent period lasted for 8 or 9 days. Best fits were obtained with the lognormal-like function, leading to a basic reproduction ratio of 5.9.

Subclinically infected pigs were investigated in this experiment. Pigs clinically affected with PMWS are reported to evidence a lower response in neutralizing antibodies than subclinically affected pigs (Meerts *et al.* 2006; Fort *et al.* 2007). Hence transmission can be expected to differ (rate and duration) between subclinically PCV2-infected and PMWS animals. It would be worth carrying out this experiment in PMWS pigs.

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