An Experimental Infection With Classical Swine Fever Virus in Pregnant Sows: Transmission of the Virus, Course of the Disease, Antibody Response and Effect on Gestation

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With 5 figures and 1 table

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Summary

An experimental infection with classical swine fever (CSF) virus in 12 conventional gilts, housed in a sow-box housing system, was conducted in order to evaluate horizontal transmission, clinical, virological and serological response, and the effect on gestation. Two of the 12 gilts, of which 10 were pregnant, were experimentally inoculated. They became viraemic for the first time 6 days post-inoculation (dpi). The contact gilts became viraemic between 18 and 21 days post-inoculation. On the basis of virological findings and the martingale estimate of $R_0$ (13.0) it was concluded that the two experimentally inoculated gilts infected all contact gilts, although random contacts between gilts were not possible. The presence of CSF infection could be diagnosed earlier and during a longer period when the leucocyte count or polymerase chain reaction were used in comparison with virus isolation in whole blood ($P < 0.05$). The observed clinical symptoms were atypical and highly variable between the gilts, which hampered clinical diagnosis. The pregnant gilts became infected between day 43 and 67 of gestation. In all cases vertical virus transmission occurred and this resulted partially in abortion and/or mummification.

Introduction

Classical swine fever (CSF) is known to be a highly contagious pig disease causing considerable economic losses. In 1980 the European Union (EU) adopted an eradication strategy for CSF (European Union, 1980). As the control of CSF in the EU is based on a policy of non-vaccination and stamping-out, this policy has resulted in an eradication of the disease in most of the member states of the EU.

However, recent outbreaks have shown that CSF epidemics in densely populated pig areas are difficult to control and can have dramatic consequences (Elbers et al., 1999). In the 1997–98 CSF epidemic in the Netherlands, it once again has been shown that the early detection of the primary CSF-infected herd is crucial to minimize the size of an outbreak. The longer that CSF remains undetected, the larger the opportunities are for the virus to become widespread (Horst et al., 1998; Elbers et al., 1999). The most important hindrance to detecting the presence of CSF infection at an early stage is the appearance of atypical
clinical symptoms and the relatively large chance of missing an infection if only a limited number of blood samples are taken (Koenen et al., 1996).

In order to design a surveillance system that maximizes the possibility of detecting the presence of infection, it is essential to have detailed information on the clinical picture and on the dynamics of the infection. Moreover, information of the within-herd virus spread is of great importance in order to assess the risk of between-herd virus spread.

The spread of CSF in weaner and slaughter pigs has already been investigated (Laevens et al., 1998, 1999). Similar experiments in sows housed in a sow-box housing system have not yet been conducted.

In the present study the transmission of CSF virus among gilts housed in a sow-box housing system was examined. Furthermore, the virological and serological response, the clinical symptoms, and the effect on gestation, following a CSF infection are described.

### Materials and Methods

#### Animals

Twelve conventional gilts, 8 months of age, originating from a selection herd and controlled for the absence of bovine viral diarrhoea (BVD) and CSF antigen and antibodies were used.

#### Virus

The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993–94 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using monoclonal antibodies, it was characterized to be similar to an isolate known as the ‘souche Lorraine’ (Koenen and Lefebvre, 1995). Virus infectiousness was $10^3$ median tissue culture infective dose (CTID$_{50}$/ml).

#### Experimental design

Upon arrival, the gilts were housed in individual sow boxes where oestrus detection was carried out on a daily basis. Within a range of 24 days oestrus was observed in all gilts. During oestrus, gilts were inseminated twice. Twenty-five days after the last insemination the gilts were checked for pregnancy using ultrasound. After pregnancy had been diagnosed the gilts were transferred to an isolation unit where they were again housed in individual sow-boxes. The two gilts that were inseminated first (longest period of gestation) were housed in boxes 3 and 10, respectively. The two gilts that were not pregnant were housed in the middle boxes (6 and 7). The remaining eight gilts were randomly allocated to the remaining boxes. Direct nose-to-nose contact was only possible between neighbouring pigs.

Following a 10-day acclimatization period after arrival at the isolation unit, the two gilts, housed in boxes 3 and box 10, were experimentally inoculated with CSF virus through a deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml). After experimental inoculation, the sows were not released from the boxes until the end of the experiment or at the moment of death. All gilts that survived the infection were slaughtered 1 week before the end of gestation.

In the 75-day post-inoculation period, the boxes were visited following a strict route starting as far as possible from the experimentally inoculated gilts and moving towards the sources of infection. By applying this visiting procedure, it was made certain that the virus was not transferred from infected to uninfected gilts during sample collection. Additionally, all materials necessary for blood sampling and rectal temperature monitoring were provided for each box separately.

#### Sample collection and clinical examination

Clotted and heparinized blood samples were collected from all gilts upon arrival. Blood samples were also taken upon arrival at the isolation unit and 2 days prior to inoculation. During the
post-inoculation period, blood samples were collected from all gilts every 3 days until 54 days post inoculation (dpi), and every 6 days between 54 and 75 dpi. Additionally, swabs of nasal secretion and faeces were collected from the experimentally inoculated gilts every 3 days during the first 30 dpi. Simultaneously with sample collection, all gilts were examined clinically. The following symptoms were recorded: liveliness (apathy), body condition (cachexia), coughing, conjunctivitis, diarrhoea, ataxia, and erythema. Rectal temperature, feed intake and mortality were recorded daily. From every pig that died or had to be killed, tissue samples (tonsil, muscles of shoulder and rump, mesenterial, ileocecal and maxillary lymph node, kidney, spleen, heart, lung, liver, brain, eye fluid, blood, faeces, urine) were collected. After death or after abortion, blood and tissue samples (tonsils, kidney, spleen, heart, and lung) were collected from the foetuses.

**Sample analyses**

For virus isolation (VI) in blood, 100 μl whole blood was inoculated in duplicate onto a non-confluent monolayer of PK15 cells cultured in multiwell plates (24 wells/plate). After 48 h, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Additionally, a single tube reverse transcriptase (RT)-nested polymerase chain reaction (nPCR) test (McGoldrick et al., 1999) was used to detect viraemia in serum. The same single tube RT-nPCR test was used to detect CSF virus in nasal secretion and faeces of the experimentally inoculated pigs.

For antibody detection in serum, the virus neutralization (VN) test and the CTB-ELISA (Ceditest) (Wensvoort et al., 1988) were used. Leucocyte count was carried out using the Coulter-Counter ZM (Analis; Coulter Electronics, Bedfordshire, England).

**Data analyses**

The basic reproduction ratio ($R_0$), a measure of transmission of infection, and defined as the mean number of new infections arising from one typical infectious case introduced in a totally susceptible population, was calculated using the martingale (de Jong and Kimman, 1994) and the maximum likelihood (Bouma et al., 1996) estimator. The martingale estimator is defined as:

$$R_{0\text{mart}} = \frac{N}{C - Z} \sum_{i=X+1}^{6} \frac{1}{i}$$

To calculate $Z$ (the sum of fraction of infectious periods that were spent at the time when no susceptibles remained), the day of infection was estimated for all gilts and it was assumed that the gilts were infectious during their entire viraemic period. The ‘SIR’ (susceptible-infective-removed) model was used to describe the final size distribution in terms of $R_{0\text{mart}}$ (de Jong and Kimman, 1994). A statistical test of $R_{0\text{mart}}$ were performed as described by Kroese and De Jong (in prep.) ($H_0$: $R_0 \leq 1$).

The maximum likelihood estimator is calculated numerically from:

$$R_{0\text{MLE}} = \max \prod_{i=1}^{n} F(X_i, R_0 | N, S_0, I_0)$$

Fever was defined as a rectal temperature $> 39.0^\circ C$. This is the one-sided upper 95 % confidence limit (CL) calculated on the average rectal temperature of each gilt during the last 3 days before experimental inoculation. Leucopenia was defined in a similar way and the one-sided lower 95 % CL limit was equal to 11 500 cells/ml.

Periods during which a given clinical symptom occurred started with the first of at least two subsequent observations of a given clinical symptom and ended with the first of at least two subsequent observations for which the given clinical symptom was absent. Periods of positive VI, PCR and leucopenia were defined in a similar way.

The time to first leucopenia, positive PCR and positive serology was compared with the time to first positive VI in blood using a paired sample $t$-test (SPSS, Chicago, USA). In addition, the period during which leucopenia was present and during which PCR was positive, was compared with the period during which VI in blood was positive using a paired sample $t$-test (SPSS).
Results

Both experimentally inoculated gilts were first detected positive for CSF on VI at 6 dpi. At the same time virus was also detected (PCR) for the first time in the nasal secretion and faeces of these gilts. The number of gilts with first positive VI, PCR and VN test at each time point is shown in Fig. 1. In gilt 7 no viraemia was detected using VI, although PCR and VN were positive.

Based on the results of VI in the experimentally inoculated gilts, the moment of infection of the contact infected gilts was estimated to be two observations (6 days) before the first positive VI. As there was no positive VI in gilt 7 the moment of infection of gilt 7 was estimated based on the results of the PCR in serum. The first positive PCR in serum occurred on average 1.64 days before the first positive VI (Fig. 2). Therefore the day of infection was estimated to be 4.36 days (6–1.64) before the first positive PCR. However, since there were only observations every 3 days, the estimated day of infection of gilt 7 was equal to one observation (3 days) before the first positive PCR.

The martingale estimate of $R_0$ was calculated to be 13.0 ($H_0: R_0 \leq 1; P < 0.01$). As no susceptible gilts remained at the end of the experiment, the maximum likelihood estimate of $R_0$ was $+\infty$. The lower boundary of the 95% CI of the $R_{\text{mle}}$ was 1.24.

In Fig. 2 the diagnostic techniques are compared, with VI in whole blood as reference. Both leucopenia (1.8 days) and positive PCR in serum (1.6 days) occurred significantly ($P < 0.05$) earlier than positive VI. Antibodies (VN test) were detected on average 6.3 days after the first positive VI ($P < 0.01$). The average period during which leucopenia was present (10.5 days) and PCR was positive (12 days) was also significantly longer ($P = 0.015$ and $P = 0.049$, respectively) in comparison with the period during which VI in whole blood (7 days) could be observed.

The clinical symptoms are summarized in Figs 3 and 4. Eight out of the 12 gilts showed fever (> 39.0°C). Fever appeared on average 5 days after infection, varying from
1 to 10 days. The duration of fever varied between 2 and 31 days. The occurrence and the duration of the other clinical symptoms were also highly variable. For example, gilt 6 remained without any clinical symptom during the whole observation period, although leucopenia and fever were observed, whereas gilts 8, 9, and 10 showed conjunctivitis and erythema without having fever. Gilts 2 and 3 died 15 and 20 days after infection, respectively (Fig. 5). They both showed severe clinical illness before dying.
On the day of experimental inoculation the inoculated gilts were both on day 55 of gestation. The other gilts were between day 31 and 55 of gestation (Fig. 1). All gilts became infected between day 43 and 67 of gestation (Fig. 5). Four of the 10 pregnant gilts aborted. The abortions occurred between 13 and 49 days after infection. In all pregnant gilts (aborted + killed) the offspring was at least partially infected (Table 1). In addition, mummification of a part of the offspring occurred (Table 1). None of the infected offspring had seroconverted against CSF.

**Discussion**

During the 1997 epidemic in The Netherlands 322 out of 429 outbreaks were detected on the basis of the presence of clinical signs (Elbers et al., 1999). This illustrates the importance of regular clinical examinations during an outbreak. However, detecting a
present CSF infection by clinical examination seems to be more difficult in breeding herds than in fattening herds. In fact, during the 1993–94 epidemic in Belgium it was found that the time between the first occurrence of clinical signs and the reporting of CSF suspicion was longer when the disease was introduced in sows, boars or suckling piglets as compared with fattening pigs (Koenen et al., 1996).

The extended time between the detection of the first clinical symptoms and the suspicion of a CSF infection in breeding herds compared to fattening herds may be the result of a combination of factors.

First, the clinical symptoms in sows, following a CSF infection, are atypical and discrete and do not immediately lead to suspicion of CSF, unless the fact that the farmers spend more time in a sow unit which makes the inspection of the sows more intense (Elbers et al., 1999). Secondly, in a sow-box housing system, virus spread may proceed more slowly, as it is generally assumed that direct contact between infected and susceptible pigs is the principal method of virus transmission (Edwards, 2000).

The atypical and discrete clinical symptoms and the low mortality rate following a CSF infection in sows are probably the most important factors causing a delayed diagnosis. In this experiment the first clinical symptoms that could be observed were fever and leucopenia. Other clinical symptoms (apathy, ataxia, conjunctivitis, constipation, cachexia) occurred later on and in a variable number of gilts. The symptoms are comparable with observations in the field during outbreaks (Koenen et al., 1996; Elbers et al., 1999). In comparison with experimental infections with the same strain in weaner and slaughter pigs (Laevens et al., 1998, 1999) clinical symptoms were less severe in gilts. This is in agreement with previous studies where it was found that the clinical course of the infection is influenced by the age of the infected animal (Depner et al., 1994; Koenen and Lefebvre, 1995; van Oirschot, 1999). It should however, be emphasized, that a large individual variability in the occurrence of the clinical symptoms was observed.

The ‘carrier–sow’ syndrome remains important in the epidemiology of CSF, especially at the beginning and the end of an outbreak when the control measures are less strict. In this experiment, some of the sows aborted after infection and others produced litters with mummified and living piglets. These findings are in comparison to those that have been described in the literature (Plateau et al., 1980; Meyer et al., 1981; Terpstra, 1988; Dahle and Liess, 1992). It is difficult to assess whether the living piglets were all viable since the sows were killed some days before the end of the gestation period. The observation that in several gilts only part of the litter was infected has also been described in previous literature (van Oirschot, 1979; Meyer et al., 1981).

The second possible explanation for the delayed diagnosis of a CSF infection in sows is the slower virus transmission in sows, especially in sow-box housing systems. The dynamics of a CSF infection in sows may differ from an infection in weaner or slaughter pigs because of the difference in age and housing system. The relation between age and the severity of the clinical symptoms has been discussed previously. However, the effect of age on the virus transmission has not been fully explained yet.

In this experiment it was found that both of the experimentally inoculated gilts became viraemic between 3 (last negative response on VI) and 6 (first positive response on VI) dpi. These results are consistent with previous experimental inoculations in weaner and slaughter pigs (Depner et al., 1994; Laevens et al., 1998, 1999; Dewulf et al., 2000) and indicate that age has no major effect on the time between infection and viraemia.

The calculated $R_{0\text{mt}}$ (13.0), which is comparable to what has been found in previous experiments for slaughter pigs (13.7) (Laevens et al., 1999), and the observation that the two experimentally inoculated gilts infected all contact gilts, indicates that the virus spread in gilts proceeds relatively rapidly. These results also demonstrate clearly that CSF virus spread is indifferent to direct nose-to-nose contact. Therefore, airborne virus transmission may be more important in a sow-box housing system than previously accepted.

In view of the atypical and variable clinical symptoms, confirmation of a suspected infection should be done by diagnostic tests. It has been shown that leucocyte count and
PCR are the two techniques that respond first, on average 2 days before the VI. Leucocyte count is a fast and easy technique that is sensitive however, but not at all specific. PCR on the other hand is sensitive and specific but it is labour intensive and expensive. To limit the workload, a first selection of the samples based on leucocyte count followed by a PCR on the samples with leucopenia may be preferred. An additional advantage of leucocyte count and PCR is that viraemia can be detected during a longer period compared to VI. The serology is of little use for an early detection, it is of great importance for screening purposes, due to the large number of samples that can be processed and due to the long detectable period.

In conclusion it can be stated that there is no major difference in the dynamics of a CSF infection between breeding and fattening pigs. Therefore, the late clinical detection of the presence of CSF infection is mainly due to the atypical and discrete clinical symptoms. As a preventive measure it may be recommended that in the presence of an unknown disease in sows, with atypical clinical symptoms as described, blood samples should be taken for CSF diagnosis. Leucocyte count with PCR as confirmation test is very suitable for an early diagnosis.

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**References**


