
J. Dewulf\textsuperscript{a,b}, H. Laevens\textsuperscript{a,b}, F. Koenen\textsuperscript{c}, K. Mintiens\textsuperscript{d} and A. de Kruif\textsuperscript{a}

\textsuperscript{a} Department of Reproduction, Obstetrics and Herd Health, \textsuperscript{b} Veterinary Epidemiology Unit, School of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium, tel: +32 9 264 75 48, fax: +32 9 264 77 98, E-mail: jeroen.dewulf@rug.ac.be

\textsuperscript{c} Veterinary and Agrochemical Research Centre, \textsuperscript{d} Department of Virology, \textsuperscript{\textit{b}} Coordination centre for veterinary diagnostics, Groeselenberg 99, B-1180 Brussels, Belgium

ABSTRACT

An experimental infection with classical swine fever (CSF) virus in twelve 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 out of the twelve gilts, of which 10 were pregnant, were experimentally inoculated. They became viraemic for the first time 6 days post inoculation (dpi). Contact gilts became viraemic between 18 and 21 days post inoculation. Based on the virological findings and the martingale estimate of $R_0$ (25.1) It can be concluded that all contact gilts got infected by the two experimentally inoculated gilts, although random contacts between gilts were not possible.

A present CSF infection could be diagnosed earlier and during a longer period when leukocyte count or PCR was 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 got infected between day 43 and 67 of gestation. In all cases vertical virus transmission occurred. This resulted partially in abortion and/or mummification.

1. INTRODUCTION

Classical swine fever (CSF) is known as a highly contagious pig disease causing considerable economic losses. In 1980 the European Union (EU) adopted an eradication strategy for CSF (Council Directive 80/217, EU). Since 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 1997-1998 CSF epidemic in the Netherlands, it once again has been proven that the early detection of the primary CSF infected herd is crucial to minimize the size of an outbreak. The longer 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 detect a present CSF infection in an early stage is the appearance of atypical clinical symptoms and the relatively large chance to miss an infection if only a limited number of blood samples are taken (Koenen et al., 1996).

In order to design a surveillance system which maximises the possibility of detecting a present 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 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; Laevens et al., 1999). Similar experiments in sows housed in a sow-box housing system have not yet been conducted.

In the presented 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.
2. MATERIALS AND METHODS

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

2.2. Virus
The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using monoclonal antibodies, it was characterised 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 ($\text{TCID}_{50}$/ml).

2.3. Experimental design
Upon arrival, 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 gilts were checked on pregnancy using ultrasound. The number of days of gestation on the day of experimental inoculation are shown in Figure 1. After pregnancy diagnosis, 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 8 gilts were randomly allocated to the remaining boxes (Figure 1). Direct nose-to-nose contact was only possible between neighbouring pigs.

2.4. Sample collection and clinical examination
Clotted and heparinized blood samples were collected from all gilts upon arrival. Again, blood samples were taken upon arrival at the isolation unit and two 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 (cachexy),...
coughing, conjunctivitis, diarrhoea, ataxia, and erythema. Rectal temperature, feed intake and mortality were recorded daily.

From every pig that died or had to be euthanatized, 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 dead or after abortion, blood and tissue samples (tonsils, kidney, spleen, heart, and lung) were collected from the foetuses.

2.5. 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 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Additionally, a single tube RT-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 neutralisation (VN) test and the CTB-ELISA (Ceditest) (Wensvoort et al., 1988) were used. Leukocyte count was carried out using the Coulter-Counter ZM (Analis).

2.6. 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 and the maximum likelihood estimator.

The martingale estimator is defined as:

$$ R_{0mrt} = \frac{N}{C - Z} \sum_{i=S_{i+1}}^{S_0} \frac{1}{i} $$

where $N$ is the total number of animals at the beginning of the outbreak, $C$ is the total number of cases that occurred during the observation period, $Z$ is the sum of fractions of infectious periods that were spent at the time when no susceptibles remained, $S_0$ is the number of susceptibles at the beginning of the observation period, and $S_t$ is the number of susceptibles at the end of the observation period (de Jong and Kimman, 1994). To calculate $Z$, 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-Infected-Removed) model was used to describe the final size distribution in terms of $R_{0mrt}$ (de Jong and Kimman, 1994). Statistical test of $R_{0mrt}$ were performed as described by Kroese and De Jong (in preparation) ($H_0$: $R_0 \Omega 1$).

The maximum likelihood estimator is calculated numerically from:

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

where $F(X_i, R_0 | N, S_0, I_0)$ is the likelihood function for the observed value $X_i$. $X$ is the total number of pigs that become infected, $N$, $S_0$, and $I_0$ are the total number of animals, the number of susceptible animals and the number of infectious animals at the beginning of the outbreak, respectively (Bouma et al., 1996).

Fever was defined as a rectal temperature > 39.0°C. This is the one-sided upper 95% confidence limit (CL) calculated on the average rectal temperature of each gilt during the last three 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). Also 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).

3. 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 Figure 2. In gilt 7 no viraemia was detected using VI, yet PCR and VN were positive.

Figure 2: Virological and serological response after infection

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.

Since 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 (Figure 3). 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 three 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 25.1 ($H_0: R_0 = 1; p<0.01$). Since 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 $R_{\text{bulk}}$ was 1.24.

In Figure 3 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.

**Figure 3: Different diagnostic methods, using virus isolation (VI) as reference**

* significantly earlier and longer than virus isolation ($p < 0.05$)
** significantly later than virus isolation ($p < 0.01$)
\[1] \text{percentage of gilts with positive test}

**Figure 4: Evolution of temperature and leukocyte count**

- Mean leucocyte count
- Mean rectal temperature
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 (Figure 1). All gilts got infected between day 43 and 67 of gestation (Figure 6). Four out of the 10 pregnant gilts aborted. The abortions occurred between 13 and 49 days after infection. In all pregnant gilts (aborted + euthanatized) the offspring was at least partially infected (between 45 and 100%). In four out of the five gilts that did not die nor abort before the end of the experiment, the offspring was partially mummified (between 20 and 73%). None of the infected offspring had seroconverted against CSF.

Figure 5: Different clinical symptoms, using beginning of viraemia (VI) as reference

1 percentage of gilts with positive test, based on observations of 11 gilts, since no viraemia (VI) was detected in gilt 7
2 percentage of gilts with positive test, based on observations of only 10 gilts, since gilt 12 showed already erythema and conjunctivitis before the day of experimental inoculation

Figure 6: Moment of infection in relation to the day of gestation

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 (Figure 1). All gilts got infected between day 43 and 67 of gestation (Figure 6). Four out of the 10 pregnant gilts aborted. The abortions occurred between 13 and 49 days after infection. In all pregnant gilts (aborted + euthanatized) the offspring was at least partially infected (between 45 and 100%). In four out of the five gilts that did not die nor abort before the end of the experiment, the offspring was partially mummified (between 20 and 73%). None of the infected offspring had seroconverted against CSF.
The clinical symptoms are summarized in Figures 4 and 5. Eight out of the twelve 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 (Figure 6). They both showed severe clinical illness before dying.

4. DISCUSSION

During the 1997 epidemic in The Netherlands 322 out of 429 outbreaks were detected based on 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 1993-1994 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 incline immediately CSF suspicion, unless the fact that the farmers spent 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 much slower, since it is generally assumed that direct contact between infected and susceptible pigs is the principal way 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, ataxy, conjunctivitis, constipation, cachexy) 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; Laevens et al., 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 emphasised, that a large individual variability in the occurrence of the clinical symptoms was observed.

The effect of the infection on the gestation as observed in this experiment is comparable to what is described in literature (Terpstra, 1988). 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.

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 experimental 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; Laevens et al., 1999; Dewulf et al., 2000) and indicate that age has no major effect on the time between infection and viraemia.

The calculated $R_{0}$ (25.1), which is larger than 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 fast. 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 leukocyte count and PCR are the two techniques that respond first, on average 2 days before the VI. Leukocyte count is a fast and easy technique that is sensitive however 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 leukocyte count followed by a PCR on the samples with leukopenia may be preferred. An additional advantage of leukocyte 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 a present 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. Leukocyte count with PCR as confirmation test is very suitable for an early diagnosis.

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6. REFERENCES


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