



HAL
open science

Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols

Eefke Weesendorp, Wil J.M. Landman, Arjan Stegeman, Willie L.A. Loeffen

► **To cite this version:**

Eefke Weesendorp, Wil J.M. Landman, Arjan Stegeman, Willie L.A. Loeffen. Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols. *Veterinary Microbiology*, 2007, 127 (1-2), pp.50. <10.1016/j.vetmic.2007.08.013>. <hal-00532307>

HAL Id: hal-00532307

<https://hal.science/hal-00532307v1>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Accepted Manuscript

Title: Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols

Authors: Eefke Weesendorp, Wil J.M. Landman, Arjan Stegeman, Willie L.A. Loeffen



PII: S0378-1135(07)00402-6
DOI: doi:10.1016/j.vetmic.2007.08.013
Reference: VETMIC 3793

To appear in: *VETMIC*

Received date: 12-6-2007
Revised date: 3-8-2007
Accepted date: 10-8-2007

Please cite this article as: Weesendorp, E., Landman, W.J.M., Stegeman, A., Loeffen, W.L.A., Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.08.013

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Detection and quantification of classical swine fever virus in air samples originating
2 from infected pigs and experimentally produced aerosols

3

4

5 Eefke Weesendorp¹, Wil J.M. Landman², Arjan Stegeman³, Willie L.A. Loeffen¹

6

7 ¹Central Institute for Animal Disease Control (CIDC-Lelystad), Wageningen UR, P.O.Box 2004, 8203

8 AA Lelystad, the Netherlands

9 ²Poultry Health Centre, Animal Health Service, P.O. Box 9, 7400 AA, Deventer, the Netherlands

10 ³Department of Farm Animal Health, Faculty of Veterinary Medicine, University of Utrecht, Yalelaan 7,

11 3584 CL Utrecht, the Netherlands

12

13

14 Corresponding author:

15 Eefke Weesendorp, Department of Virology

16 Central Institute for Animal Disease Control (CIDC-Lelystad)

17 P.O.Box 2004

18 8203 AA Lelystad, the Netherlands

19 Tel.: 0031-320-238677

20 Fax.: 0031-320-238668

21 E-mail: eefke.weesendorp@wur.nl

22

23

24

25

26

27

28

29

30

31 Abstract

32 During epidemics of Classical Swine Fever (CSF) neighbourhood infections occurred where
33 none of the 'traditional' routes of transmission like direct animal contact, swill feeding,
34 transport contact or transmission by people could be identified. A hypothesized route of virus
35 introduction for these herds was airborne transmission. In order to better understand this
36 possible transmission route, we developed a method to detect and quantify classical swine
37 fever virus (CSFV) in air samples using gelatine filters. The air samples were collected from
38 CSFV infected pigs and after experimental aerosolization of the virus. Furthermore, we studied
39 the viability of the virus with time in aerosolized state. Three strains of CSFV were aerosolized
40 in an empty isolator and air samples were taken at different time intervals. The virus remained
41 infective in aerosolized state for at least 30 min with half-life time values ranging from 4.5 to 15
42 min. During animal experiments concentrations of $10^{0.3}$ TCID₅₀/m³ to $10^{1.6}$ TCID₅₀/m³ CSFV were
43 detected in air samples originating from the air of the pig cages and $10^{0.4}$ TCID₅₀/m³ to $10^{4.0}$
44 TCID₅₀/m³ from the expired air of infected animals. This is the first study describing the
45 isolation and quantification of CSFV from air samples originating from infected pigs and their
46 cages, supporting previous findings that airborne transmission of CSF is feasible.

47

48 Key words: Aerosol; Air sampling, Airborne transmission, Classical swine fever virus, Quantification

49

50 Introduction

51 During epidemics of classical swine fever (CSF) the route of virus introduction into a farm is often
52 unclear (Koenen *et al.*, 1996; Elbers *et al.*, 1999). If newly infected farms with an unknown route of
53 virus introduction are located within a 1 km radius of a previously infected farm, they are usually
54 classified as neighbourhood infections, because farms within this radius have a significantly higher risk
55 of infection than other farms (Laevens, 1999; Stegeman *et al.*, 2002). Although underreporting of well-
56 known dangerous contacts, such as live pigs, swill feeding, transport contact or transmission by
57 people may be a reason for being unable to elucidate the route of virus introduction, (Elbers *et al.*,
58 2001), alternative routes of virus introduction have also been hypothesized for these neighbourhood
59 infections. These included introduction by arthropods, birds, pets and rodents (Stewart *et al.*, 1975;
60 Terpstra, 1987; Dewulf *et al.*, 2001; Kaden *et al.*, 2003), but also airborne spread through aerosols

61 produced by infected animals (Terpstra, 1987; Laevens *et al.*, 1999; Dewulf *et al.*, 2000; Ribbens *et*
62 *al.*, 2004). Even aerosols produced during high-pressure cleaning of the electrocution equipment, used
63 to kill the pigs on a neighbouring infected herd, were taken into consideration (Elbers *et al.*, 2001).

64 Moreover, Laevens (1999) concluded on the basis of studies of the Belgian CSFV epizootic of
65 1993-1994 that the likelihood of a neighbouring herd to become infected was, among others,
66 dependent on the frequency with which it was downwind of the primary infected herd. In contrast,
67 Crauwels *et al.* (2003), using data of the Dutch epidemic of 1997-1998, could not associate new
68 infections with the prevailing direction of the wind. Instead, they observed a decreasing infection rate
69 with increasing radial distance from the primary infected herd.

70 Experimental studies showed that CSFV can be transmitted between groups of pigs that are
71 not in direct contact (Hughes and Gustafson, 1960; Terpstra, 1987; Laevens *et al.*, 1998; Laevens *et*
72 *al.*, 1999; Dewulf *et al.*, 2000; González *et al.*, 2001). Likely the mechanism of virus transmission is by
73 air. Animals can generate aerosols containing virus after sneezing and coughing or during normal
74 breathing. Also splashes of urine and faeces or sources like bedding and feed can generate aerosols
75 (Stärk, 1999). However, until now to our knowledge no reports on the successful isolation of CSFV
76 from the air have been published. Terpstra (1987) was able to induce indirect transmission of CSFV
77 between infected donor pigs and susceptible recipients, but his attempts to isolate the virus from air
78 samples using both an all-glass cyclone and a May three stage liquid impinger were unsuccessful
79 (May, 1966; Terpstra, unpublished, 1986). Also using polyethersulfon membrane filters isolation of
80 CSFV from the air of rooms housing experimentally infected pigs failed (Stärk, 1998). These failures
81 were explained by a low excretion rate of CFS virus in aerosols by the infected animals and/or a lack
82 of sensitivity of their sampling system and/or virus detection method. As a consequence, the amount
83 of virus shed by infected pigs in the air is still unknown. This knowledge, combined with knowledge of
84 the virus survival in aerosols, is however of great value in understanding the contribution of airborne
85 transmission in general and to neighbouring herds more specifically.

86 In this paper we describe the detection and quantification of CSFV in air samples originating
87 from infected pigs and experimentally produced aerosols. The aerosolized CSFV was studied using an
88 air sampling technique with gelatine filters and the MD8 airscan, as it proved to be successful at the
89 isolation of a number of airborne pathogens affecting poultry such as *Mycoplasma gallisepticum*, *M.*
90 *synoviae*, *Enterococcus faecalis*, Newcastle disease virus and Gumboro disease virus (Aarnink *et al.*,

91 2004; Landman and van Eck, 2001; Landman *et al.*, 2004). After aerosolization the initial loss and the
92 viability of the virus with time were analyzed. Next, shedding rates of CSFV in air samples originating
93 from infected pigs and their cages were studied.

94

95 **Materials and Methods**

96 *Viruses*

97 Three CSFV strains were used in the experiment: the highly virulent Brescia strain (genotype 1.2,
98 derived from a strain obtained from Brescia, Italy, 1951), the moderately virulent Paderborn strain
99 (genotype 2.1, isolated in 1997 during the outbreak in the Paderborn area of Germany) and the low
100 virulent strain Zoelen (genotype 2.2, originally isolated from pigs in a Dutch farm in 1974). According to
101 the classification of CSFV strains by Van Oirschot (1988) infection with a highly virulent strain results
102 in death of nearly all pigs, infection with a moderately virulent strain results in subacute or chronic
103 illness leading to death or recovery while pigs infected with a low virulent strain show few or no signs
104 of disease and recover from the infection.

105

106 *Experimental aerosol production*

107 Virus stocks for aerosolization were prepared in cell culture medium (Eagle minimum essential
108 medium (EMEM) supplemented with 10% antibiotics and 10% fetal bovine serum (FBS)). One ml of
109 the Brescia and Paderborn virus stocks contained approximately 10^5 TCID₅₀ (tissue culture infectious
110 dose 50%), the Zoelen virus stock contained approximately 10^6 TCID₅₀, as determined by virus
111 titration.

112 The three different CSFV strains were aerosolized successively in an empty isolator (Beyer &
113 Eggelaar, Utrecht, the Netherlands) with a volume of 0.924 m³. An air compressor (Compact 102/3
114 OF, Creemers compressors, Eindhoven, the Netherlands) was coupled to a spray-head (Walther Pilot
115 I spray-head with 0.5 mm diameter; Walther Spritz- and Lackiersysteme, Wuppertal, Germany) to
116 generate the aerosols. A volume of 10 ml of virus stock was aerosolized in 95-180 seconds at a
117 pressure of 2 bar. The isolator temperature was 21-22°C and the relative humidity after aerosol
118 production was 60-74%. Aerosolization of each virus and for each sampling protocol (see below) was
119 carried out in duplicate. After each aerosol experiment the ventilation, with a rate of 1324 m³/h, was

120 switched on for 15 min before the next aerosolization. Control air samples were taken to ensure that
121 virus concentrations were reduced to a level below the detection limit.

122

123 *Characterization of the aerosol spectrum*

124 The droplet size distribution of the aerosol, produced by the Walther Pilot I spray-head, was
125 determined with a laser diffraction particle size analyzer (Mastersizer-S long bed; Malvern Instruments
126 Ltd., Malvern, UK). The test was performed in triplicate as described by Landman *et al.* (2004). Briefly,
127 ten ml of demineralised water were aerosolized in 120 seconds at a pressure of 2 bar. The aerosol
128 spectra were determined holding the spray-head 4 cm distant to the laser beam and approximately 2
129 cm from the lens. A 300 mm lens (measuring range: 0.5 – 990 μm) with open bench was used. The
130 temperature during the experiments was 22.8°C and the relative humidity 45.8%.

131

132 *Air sampling after experimental aerosol production*

133 During the aerosol experiments the concentration of CSFV (per m^3 air) with time was studied. The air
134 was sampled immediately after aerosol production to determine the initial loss, and then after 15 and
135 30 min to calculate the half-life time values of the airborne virus. Samples were taken using the MD8
136 airscan sampling device (Sartorius, Nieuwegein, the Netherlands) and sterile gelatine filters of 3 μm
137 pore size and 80 mm diameter (type 17528-80-ACD; Sartorius). Two different sampling protocols were
138 used, sampling with an air speed of 2 m^3/h for 2 min or with an air speed of 8 m^3/h for 10 min. After
139 sampling, the filters were dissolved in 5 ml of medium (EMEM supplemented with 10% antibiotics and
140 5% FBS) kept at 37°C. Virus titration was used to determine virus concentrations.

141 The detection limit of the air sampling procedure varied depending on the amount of air
142 sampled and was calculated as demonstrated next for the 2 m^3 of air/h during 2 min sampling
143 protocol. In order to obtain a positive result in the virus titration (performed in fourfold), at least one of
144 the four wells containing the undiluted sample should be tested virus positive. The corresponding virus
145 titer is $10^{0.65}$ TCID₅₀/ml. As 67 liters of air were sampled and the filter was dissolved in 5 ml, the
146 detection limit was $10^{2.52}$ TCID₅₀/m³ air. If sampling was performed for 10 min at 8 m^3/h , the detection
147 limit was $10^{1.22}$ TCID₅₀/m³ air.

148 The half-life time value of the virus in the aerosols (the time required for the quantity of
149 infectious virus to be reduced to half of its initial value) was calculated using the formula:

$$150 \quad t_{1/2} \equiv \frac{(\log_{10} 2) \times T}{\log_{10} \frac{C}{E}}$$

151 T = time interval in min

152 C = virus concentration at the start (t 0)

153 E = virus concentration after 15 or 30 min (t 15 or t 30)

154 During the 8 m³/h sampling protocol 1333 l of air were extracted from the isolator, which
 155 resulted in a too high underpressure. Therefore, the air inlet of the isolator was left open to allow the
 156 entrance of the same amount of air as was detracted with sampling. Hence, the virus concentrations
 157 obtained with this sampling protocol had to be corrected. The corrections were made assuming that
 158 the dilution, due to the air that was allowed to enter passively into the isolator, was linear. The original
 159 concentrations were calculated using the formula:

$$160 \quad C_o \equiv C_e \times \left(\frac{V_o + V_e}{V_o} \right)$$

161 C_o = original concentration

162 C_e = end concentration; concentration in the air sample obtained by virus titration

163 V_o = original volume of the isolator (924 l)

164 V_e = extracted amount of air

165

166 *Effect of the gelatine filter on the viability of CSFV*

167 To determine if the gelatine filters have a negative effect on the viability of the captured virus particles,
 168 a validation was carried out. Gelatine filters were placed in Petri dishes and inoculated with 0.5 ml of
 169 the same virus stock that was used for the aerosolization (containing 10⁵ TCID₅₀/ml of the Brescia or
 170 Paderborn strain and 10⁶ TCID₅₀/ml of the Zoelen strain). Three filters were assayed for each CSFV
 171 strain. The first filter was dissolved 30 min after inoculation, in a total of 50 ml of medium. This was
 172 done by initially adding 20 ml of medium to the filter (EMEM supplemented with 10% antibiotics and
 173 5% FBS, kept at 37°C). After placing the Petri dishes for 5 min at 37°C and gently mixing, the fluid was
 174 pipetted into flasks containing another 30 ml of medium. These 30 min represented the time interval of
 175 transport from the isolator to the laboratory. The second filter was dissolved 5 min after inoculation, in
 176 the same way as the first filter. The third filter was also dissolved 5 min after inoculation, however, only
 177 in 5 ml of medium, in order to get a higher concentration of virus in the sample. As control, the virus

178 stocks were kept for the same time period at room temperature as the filters and were diluted in the
179 same volumes of medium. Virus titration and Real-Time Reverse Transcription Polymerase Chain
180 Reaction (RRT-PCR) were used to determine virus concentrations.

181

182 *Experimental animals and design*

183 Three animal experiments were conducted with eight-week-old male pigs, obtained from a
184 conventional, but pestivirus free pig herd in the Netherlands. In each experiment five pigs were
185 inoculated with either the highly virulent Brescia, moderately virulent Paderborn, or low virulent Zoelen
186 strain. Samples were taken from the expiration air of the pigs and from their cages at different time
187 points to determine shedding rates of CSFV by virus titration and RRT-PCR.

188

189 *Housing of animals*

190 Pigs were individually housed in cages in one isolation unit. Each cage was placed in a pen separated
191 by solid walls. The cages were designed to separately collect faeces and urine for CSF studies.
192 Faeces were collected in plastic bags attached to the pigs with a Velcro system, while urine was
193 collected in a tray underneath the cage. Each time before cage air sampling was started, a cover was
194 placed on top of the cage. This resulted in an air flow from the front side of the cage to the back where
195 the MD8 was placed. The MD8 was attached to the cage with a PVC tube of 10 cm to prevent pigs
196 from touching the filter.

197

198 *Inoculation of animals*

199 Pigs were inoculated intranasally with a dose of 10^2 TCID₅₀ CSFV strain Brescia, 10^5 TCID₅₀ strain
200 Paderborn or 10^5 TCID₅₀ strain Zoelen, according to the standard infection models used in our
201 institute. One ml of the virus suspension was administered per animal (0.5 ml per nostril). The inocula
202 were back titrated to confirm the dose administered.

203

204 *Sampling procedures*

205 At day 6 and 8 post inoculation the expiration air was sampled from pigs infected with the Brescia
206 strain, at day 9, 13, 15 and 33 post inoculation from the pigs infected with the Paderborn strain, and at
207 day 7 and 13 post inoculation from the pigs infected with the Zoelen strain. Sampling was performed

208 on moments when maximum amounts of virus in leucocytes and oropharyngeal swabs were expected.
209 Thus, different sampling schemes were used for the different virus strains. During sampling the
210 gelatine filter was kept at a distance of approximately 10 cm of the pig's nose. Most animals were
211 laying and were breathing quietly. Pigs that did not lay down (the ones infected with the Zoelen strain)
212 were restrained in order to avoid touching the filter. The expired air was sampled with an air speed of 2
213 to 5 m³/h during 2 to 5 min. Air speed and sampling time were recorded. At day 5 post inoculation
214 cage air was sampled from pigs infected with the Brescia strain, and at days 9, 13 and 15 post
215 inoculation of the pigs infected with the Paderborn strain. The cage air was sampled at an air speed of
216 8 m³/h during 10 min. Filters were dissolved in 5 ml of medium (EMEM supplemented with 10%
217 antibiotics and 5% FBS) kept at 37°C. Virus concentration was determined by virus titration and RRT-
218 PCR.

219 Every two days samples were collected from oropharyngeal fluid and three times a week from EDTA
220 stabilized blood, which were analysed in the virus isolation (VI) assay. Oropharyngeal swabs were
221 weighed before and after sampling to be able to calculate TCID₅₀ per gram oropharyngeal fluid
222 (TCID₅₀/gr). The oropharyngeal fluid was suspended in 4 ml medium. After centrifugation (2500 **g** for
223 15 minutes) the samples were stored at -70°C. Leucocytes were isolated from EDTA stabilized blood
224 by adding 2 ml of EDTA blood to 4 ml 0.84% NH₄CL solution. After 10 minutes the samples were
225 centrifuged at 1000 **g** and washed twice with PBS. The pellet was resuspended in 2 ml medium and
226 stored at -70°C.

227

228 *Virus isolation and titration*

229 A volume of 125 µl of the air samples or leucocytes were incubated on a monolayer of SK6 cells
230 (permanent porcine kidney cell line) in a 24-wells plate (Greiner) at 37°C in an atmosphere with 5%
231 CO₂ for four days. A volume of 250 µl of oropharyngeal fluid was incubated for one hour on a
232 monolayer of SK6 cells. After a wash procedure, medium was added to the wells and incubated for
233 four days. After being fixated and washed, the monolayers were stained by the immuno-peroxidase
234 technique (Wensvoort *et al.*, 1986) and examined for stained cells. Virus positive air samples were
235 titrated in fourfold after making five decimal dilutions (10⁻¹ to 10⁻⁵) from the filter solution. Virus titers
236 were calculated as TCID₅₀ using the Spearman-Kärber method (Finney, 1978).

237

238 *Real-Time Reverse Transcription Polymerase Chain Reaction (RRT-PCR)*

239 RNA isolation was performed by pipetting 200 µl of the sample manually into MagNA Pure sample
240 cartridges (Roche Applied Science, Mannheim, Germany). In each run three negative samples (PBS)
241 and three dilutions of a positive control sample were included which were spread throughout the
242 cartridge. The RNA was extracted with the Total Nucleic Acid Isolation Kit (Roche Applied Science,
243 Mannheim, Germany) according to the manufacturer's instructions using the automated MagNA Pure
244 LC instrument (Roche Applied Science, Mannheim, Germany). After the MagNA Pure completed the
245 RNA isolation, the nucleic acids were removed from the MagNA Pure LC and immediately processed
246 for the RRT-PCR or stored at -70 °C in the sample cartridge until the RRT-PCR was carried out.

247 The RRT-PCR was performed with a LightCycler (LC) instrument (Roche Applied Science, Mannheim,
248 Germany) using the RNA Master Hybridization Probes Kit, as described by Van Rijn *et al.* (2004).
249 Crossing points (Cp) values were determined for each sample. The Cp value is the cycle number at
250 which the fluorescence emission from a RRT-PCR reaction rises above the background signal. A low
251 Cp value indicates high template amount, while a high Cp indicates a low template amount.

252 Filter samples from the animal experiment were tested in four-fold, as low RNA levels were suspected.
253 Samples were considered positive in case one or more test results showed a RRT-PCR reaction rising
254 above the background signal.

255

256 *Correlation between virus titration and RRT-PCR*

257 The correlation between Cp value and virus titration was determined. This enabled the quantification
258 of the viral load (TCID₅₀ per m³) represented by Cp values. Filters were dissolved in 5 ml of medium
259 and a twofold (10^{-0.3}) and tenfold (10⁻¹ to 10⁻⁵) dilutions were prepared from the Brescia, Paderborn
260 and Zoelen strain. Samples were stored at -70°C until analysis by virus titration and RRT-PCR.

261

262 *Statistical analysis*

263 The effect of the gelatine filter on the viability of the virus was analysed using a paired-sample T-test
264 (SPSS 12; SPSS Inc., Chicago, Illinois). Therefore, the differences in virus titration and RRT-PCR
265 value between the filter and stock solution were compared. *p*-values < 0.05 denoted a significant
266 difference in virus viability between the filter and stock solution.

267

268 **Results**269 *Effect of the gelatine filter on the viability of CSFV*

270 The effect of the gelatine filter on the viability of the virus was studied by comparing it with the stock
271 solution, which was given the same treatment (Table 1 and 2). The filter and stock solutions for the
272 different CSFV strains showed comparable results in both the virus titration ($p=0.56$) and the RRT-
273 PCR ($p=0.73$), although there may be a small effect on the viability of the virus with increased time
274 before processing. Processing the filter thirty minutes after inoculation resulted in a very small
275 reduction in the VI titers compared to processing five minutes after inoculation.

276 In addition, it was possible to dissolve the filter in only 5 ml of medium without negative effect on the
277 virus, as in general $1 \log_{10}$ TCID₅₀/ml higher titers were observed compared to filters dissolved in 50
278 ml. Because of this, a higher virus concentration in the sample could be obtained.

279

280 *Characterization of the aerosol spectrum*

281 The particle size distribution (D) is expressed as a volume diameter below which a certain percentage
282 of the particle volume is contained. The particle diameter below which 10% of the particle volume is
283 contained; $D(v, 0.1)$, was 6.52 μm . A diameter of 24.43 μm was found for $D(v, 0.5)$ (below which 50%
284 of the particle volume is contained) and a diameter of 49.55 μm for $D(v, 0.9)$. The average droplet size
285 distribution of three aerosol spectra is shown in Figure 1. In previous experiments (data not shown)
286 different types of medium were tested during aerosol spectrum analysis of the spray head with similar
287 results to those achieved with demineralized water. Therefore, the reflected aerosol spectrum was
288 considered representative for the medium used in this experiment (EMEM supplemented with 10%
289 antibiotics and 10% FBS).

290

291 *Assessment of the yield and viability of CSFV in air samples from experimental aerosols*

292 Three different CSFV strains were aerosolized in duplicate (experiments 1 and 2) and the
293 concentration of the CSFV was studied by means of two different sampling protocols; sampling for 2
294 min at 2 m^3/h (Table 3) or sampling for 10 min at 8 m^3/h (Table 4). When sampling was performed
295 directly after aerosolization at an air speed of 8 m^3/h , the filters clogged before the sampling time was
296 completed. This was due to the high relative humidity after aerosol generation in combination with the

297 high air speed. As in these cases less air was sampled, the results of the virus titration were corrected
298 accordingly.

299 The reproducibility of the aerosols and sampling results were good, with only small differences
300 between experiments 1 and 2. Before each aerosol experiment and after ventilation control air
301 samples were taken in which no CSFV was detected.

302 The initial loss was determined by taking air samples immediately after aerosol generation and
303 comparing it with the aerosol virus dose. These losses were similar for both sampling protocols and
304 were on average $10^{1.1}$ TCID₅₀/m³ for the Brescia strain, $10^{0.5}$ TCID₅₀/m³ for the Paderborn strain and
305 $10^{0.8}$ TCID₅₀/m³ for the Zoelen strain. Fifteen and thirty min after the aerosol production concentrations
306 had decreased, with slightly lower concentrations for the 10 min at 8 m³/h protocol than the 2 min at 2
307 m³/h protocol, except for the first experiment of the Zoelen strain.

308 The half-life time values, which give an indication of the viability of the virus with time, ranged
309 between 4.5 and 12 min. The half-life time values for the Brescia and Paderborn strain determined at t
310 30 min when sampling during 2 min at 2 m³/h were slightly higher than when sampling was performed
311 with the other protocol. The half-life time values for the Zoelen strain determined at this time point
312 were slightly higher when sampling was performed with the 10 min at 8 m³/h protocol.

313

314 *Clinical signs and virus isolation on oropharyngeal swabs and leucocytes*

315 Pigs infected with the Brescia strain showed severe clinical signs and fever (>40°C) at all air sampling
316 moments. Virus isolation on oropharyngeal swabs was positive at all air sampling moments (on
317 average $10^{4.5}$ TCID₅₀/gr at day 6 and $10^{5.6}$ TCID₅₀/gr at day 8 post inoculation), as was virus isolation
318 on leucocytes (Table 5).

319 All pigs infected with the Paderborn strain showed mild to severe clinical signs of CSF after
320 inoculation. At day 9 post inoculation fever was detected in pig number 10, and clinical signs in all
321 animals except pig number 8. At days 13 and 15 post inoculation fever was detected in pigs 6 and 10,
322 and clinical signs in pigs 6, 9 and 10. At day 33 post inoculation fever was detected in pig 6, and
323 clinical signs in pigs 6 and 10. Virus isolation on oropharyngeal swabs and leucocytes was positive at
324 all sampling moments, except for pig 8 at day 16 post inoculation (Table 6). Pig 6 and pig 10 were
325 chronically infected (more than 30 days virus positive). Virus concentrations in oropharyngeal swabs

326 from these pigs were higher (on average $10^{6.3}$ TCID₅₀/gr from day 8) than in the oropharyngeal swabs
327 from the other three pigs (on average $10^{2.9}$ TCID₅₀/gr between day 8 and 16 post inoculation).
328 Amongst pigs infected with the Zoelen strain only pig number 12 showed fever at day 7 post
329 inoculation. None of the pigs showed fever at day 13 post inoculation. Mild clinical signs were only
330 observed at day 7 post inoculation in pigs number 11, 12 and 13. Virus isolation on oropharyngeal
331 swabs from all pigs was positive at day 6 post inoculation (on average $10^{2.2}$ TCID₅₀/gr between day 6
332 and 8 post inoculation), as was virus isolation on leucocytes (Table 7). At day 13 post inoculation,
333 when the second air sampling was performed, only one pig tested positive in the VI on oropharyngeal
334 swabs and leucocytes.

335

336 *Virus isolation and RRT-PCR on air samples*

337 Air samples from cages housing Brescia infected pigs at day 5 post inoculation resulted in three RRT-
338 PCR positive samples. All pigs had RRT-PCR positive expiration air samples at day 8 post inoculation.
339 One air sample was also virus titration positive with a virus titer of $10^{3.5}$ TCID₅₀/m³ (Table 5).
340 Paderborn infected pigs yielded two RRT-PCR positive cage air sample at day 9, three at day 13, and
341 two at day 15 post inoculation. One expiration air sample from an infected pig was RRT-PCR positive
342 at day 13 and two at day 33 post inoculation (Table 6). The positive air samples mainly originated from
343 pigs 6 and 10, which were chronically infected and excreted high amounts of virus in oropharyngeal
344 swabs. None of the expiration air samples from pigs infected with the Zoelen strain were virus positive
345 (Table 7).

346

347 *Correlation virus titration and RRT-PCR*

348 A good correlation was observed between the virus titration and the RRT-PCR technique ($R^2=0.993$
349 for the Brescia strain, $R^2=0.985$ for the Paderborn strain and $R^2=0.991$ for the Zoelen strain). Based on
350 this correlation the samples of cage air had calculated virus titration titers of $10^{0.3}$ TCID₅₀/m³ to $10^{1.6}$
351 TCID₅₀/m³ for the Brescia infected pigs, and titers of $10^{0.3}$ TCID₅₀/m³ to $10^{1.4}$ TCID₅₀/m³ for the
352 Paderborn infected pigs (Tables 5 and 6). The expiration air samples from the infected pigs showed
353 calculated virus titration titers of $10^{0.7}$ to $10^{4.0}$ TCID₅₀/m³ for the Brescia infected pigs, and titers of $10^{0.4}$
354 TCID₅₀/m³ to $10^{2.2}$ TCID₅₀/m³ for the Paderborn infected pigs(Tables 5 and 6). None of the air samples
355 from the Zoelen infected pigs were positive in the RRT-PCR.

356

357 **Discussion**

358 This is the first study describing the successful detection of CSFV in air samples originating from
359 infected pigs. Virus was detected in air samples representing cage air, as well as expiration air.
360 Furthermore, in artificially created aerosols, it was shown that the virus can remain infective for at least
361 30 min after aerosol generation with half-life time values ranging from 4.5 to 15 min. The air sampling
362 device and protocols used were suitable to enable the detection and quantification of the virus in
363 mentioned air samples.

364 We were able to detect CSFV in air samples obtained from pigs infected with the Brescia or
365 the Paderborn strain, however, not from pigs infected with the low virulent Zoelen strain. This was not
366 unexpected, as in general, looking at other secreta and excreta, pigs infected with the Zoelen strain
367 excreted much lower quantities of virus than pigs infected with the Brescia and Paderborn strain (data
368 not shown). Transmission of CSFV by the air seems therefore more likely to occur during outbreaks
369 caused by highly and moderately virulent strains than by low virulent strains.

370 Classical swine fever virus shedding rates of pigs in aerosols were low compared to pigs
371 infected with for instance foot-and-mouth disease virus. The CSFV infected pigs excreted up to $10^{5.2}$
372 TCID₅₀ of infectious virus per day in expiration air while foot-and-mouth disease infected pigs excrete
373 up to $10^{8.6}$ TCID₅₀ per day (Donaldson and Alexandersen, 2002). The minimal infective dose that
374 results in fatal disease after inoculation with the highly virulent strain "Alfort" is 10 TCID₅₀ per pig
375 (Liess, 1987). For the Brescia virus strain a pig ID₅₀ of 74 TCID₅₀ after intranasal inoculation was found
376 by Terpstra (C.Terpstra, unpublished, 1984 and 1987). Based on the intranasal dose it can be
377 concluded that a sentinel pig, exposed to the expired air of an infected pig, receives a dose that is
378 most likely sufficient to induce infection. Whether this is also enough to transmit the virus over larger
379 distances (e.g. farms in the neighbourhood of an infected farm) will depend on additional factors.
380 These factors may include total virus excretion by a population of infected animals (which in turn
381 depends on number of infected animals, and virus type), weather conditions, ventilation rate, etc.
382 Further research into these parameters will be necessary to move forward with respect to the question
383 of neighbourhood infections caused by aerosols.

384 It has been shown previously that the RRT-PCR test is more sensitive than the VI technique
385 (Van Rijn *et al.*, 2004). This was confirmed in the present study as many air samples from the animal

386 experiments, negative at VI, showed positive RRT-PCR results. Besides this higher sensitivity of the
387 RRT-PCR, negative VI results could be due to loss of infectivity of virus particles. The sample taken
388 from the expired air of pig 2 at day 8 post inoculation was the only sample positive in both the virus
389 titration and RRT-PCR assay. The calculated virus titre in TCID₅₀ from the RRT-PCR result was
390 approximately 0.5 log₁₀ TCID₅₀ higher than the titer that was determined in the virus titration assay.
391 Therefore, the calculated titres of excretion rates based on PCR-results could be slightly
392 overestimated.

393 On the other hand, shedding rates of virus in aerosols by infected pigs under field circumstances is
394 expected to be higher than determined in this study. Faeces were collected separately and the
395 isolation unit had a low pig density in combination with a high ventilation rate, so subsequently dust
396 and aerosol production was low. To assess the risk of airborne transmission, further research is
397 necessary under circumstances mimicking the situation in the field, where the herein described air
398 sampling system seems a suitable tool to determine shedding rates.

399 The validation studies showed that the gelatine filter processing method did not have an
400 adverse effect on the yield and viability of the virus. Even 30 min after inoculating the virus on the filter,
401 which was the elapsed time between air sampling and processing in the aerosol experiments, no
402 significant inactivation was observed.

403 Between the different aerosolization and sampling experiments in the isolator, the isolator was
404 not decontaminated. However, it was ventilated at a high rate (1324 m³/h) for 15 min, which will have
405 reduced virus concentrations to a level far below the detection limit. This was confirmed by the
406 negative virus titration and RRT-PCR results of air samples taken before the start of each experiment
407 and directly after ventilation.

408 After generating virus containing aerosols, these aerosols are subject to both biological and physical
409 decay. Biological decay includes factors that affect the infectivity of the virus, while the physical decay
410 depends on the time particles remain suspended which is influenced by particle size and particle
411 deposition (Stärk, 1999). Directly after generating the aerosols air samples were taken and the initial
412 loss was determined. Beside the biological and physical loss the initial loss is dependent on the ability
413 of the air-sampling system to recover airborne particles. The initial losses were maximum 10^{1.66}
414 TCID₅₀/m³. In case the inability of the sampling system to recover the particles is totally responsible for
415 the initial losses, only 2.2% of the infectious particles were recovered from the air. However, the

416 manufacturer claims high absolute retention rates for micro-organisms and viruses, like 99.9995% for
417 *Bacillus subtilis niger* spores and 99.94% for T3 coliphages. Therefore, the represented data are
418 unlikely to be underestimated.

419 The physical decay after generation of the aerosols was expected to be similar in all
420 experiments as the same aerosol generator and isolator were used and climatic conditions did not
421 change. However, the highly virulent Brescia strain seems more susceptible to aerosolization or
422 sampling than the moderately and low virulent Paderborn and Zoelen strain, as shown by higher initial
423 losses and shorter half-life time values than the Paderborn and Zoelen strain. It is not clear whether
424 these differences are reproducible and significant because of the limited number of observations. Any
425 difference could be caused by differences in their susceptibility to shear forces of the spraying device,
426 to droplet evaporation and differences in susceptibility of the virus to the stress caused by the
427 sampling technique.

428 Sampling at higher air speed (8 m³/h for 10 min) resulted for the Brescia and Paderborn strain
429 in slightly lower half-life time values. The higher air speed could have caused mechanical stress to the
430 virus adhered to the filter and favoured inactivation by enhancing evaporation. Furthermore, during
431 sampling at 8 m³/h the air inlet was left open and it was assumed that the air entering the isolator
432 mixed adequately, however, in case the entering and isolator air did not mix well, fresh air could have
433 been sampled in relatively higher amounts, yielding lower half-life time values. As the same conditions
434 were applied when sampling the Zoelen strain, this explanation is however less likely.

435 Schwarte and Mathews (1954) demonstrated that aerosols of powdered lyophilized CSFV
436 obtained from blood can infect susceptible pigs up to 30 min after aerosolization. The current study not
437 only confirmed that the virus can remain infective in aerosols for at least 30 min after aerosol
438 generation, but also gives information about the half-life time values of the virus in aerosols which can
439 be used to assess the risk of airborne transmission during an outbreak of CSFV. In the present work,
440 the aerosol fluids were however prepared from virus in medium containing 10% fetal calf serum,
441 known to protect the virus. This may also have been the case in the study of Schwarte and Mathews,
442 where masses of desiccated serum and blood cells could have prevented inactivation of the virus.
443 Therefore, the infectivity of the virus in aerosols produced after sneezing and coughing or in splashes
444 from faeces or urine could be different.

445 The aerosols produced in the current study could be used to infect pigs in further experimental
446 studies. If a susceptible pig of 25 kg would be exposed during 30 min to the aerosols, assuming the
447 decrease in concentration of CSFV is linear, the uptake of virus from the Brescia, Paderborn or Zoelen
448 strain would be $10^{4.7}$ TCID₅₀, $10^{5.1}$ TCID₅₀ or $10^{6.0}$ TCID₅₀, respectively. This is based on reported tidal
449 air volumes of 9.27 to 15 l/min for 25 kg pigs with a mean respiratory rate of 35 breaths per minute
450 (reviewed by Stärk, 1999; Alexandersen and Donaldson, 2002). These calculations were made
451 assuming that not only the part of the aerosol that reaches the bronchi and lungs (with particle sizes
452 <6 µm), but also larger particles deposited in the upper airways and oro-nasal cavity will result in
453 infection (Sellers and Parker, 1969). Based on the intranasal dose it can be concluded that the
454 aerosols in this study contain a sufficient virus dose to infect pigs.

455 In conclusion, although the possibility to infect pigs with aerosols containing CSFV has been
456 demonstrated in the past, and airborne transmission has been shown in experimental studies, there
457 was no information available on the shedding rates of CSFV from infected pigs and half-life time
458 values of different aerosolized CSFV strains. These data have been generated in the present study
459 after developing an air sampling technique, which might be used to unravel the role of airborne
460 transmission in outbreaks of CSFV.

461

462 **Acknowledgements**

463 The authors thank E.A. Corbanie for performing the aerosol spectra analyses. Sjaak Quak, Piet de
464 Vegt and Ntsiki Held are thanked for their technical assistance and Armin Elbers and Eugène van
465 Rooij for critical reading the manuscript.

466

467 **References**

468 Aarnink, A.J., Landman, W.J., Melse, R.W., De Gijzel, P., Thuy, A.H., Fabri, T, 2004. Voorkomen van
469 verspreiding van ziektekiemen en milieu-emissies via luchtreiniging. Agrotechnology and Food
470 Innovations Report, Wageningen, the Netherlands.

471

472 Alexandersen, S., Donaldson, A.I., 2002. Further studies to quantify the dose of natural aerosols of
473 foot-and-mouth disease virus for pigs. *Epidemiol. Infect.* 128, 313-323.

474

- 475 Crauwels, A.P., Nielen, M., Elbers, A.R., Stegeman, J.A., Tielen, M.J., 2003. Neighbourhood infections
476 of classical swine fever during the 1997-1998 epidemic in the Netherlands. *Prev. Vet. Med.* 61, 263-
477 277.
- 478
- 479 Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A., 2000. Airborne transmission of classical
480 swine fever virus under experimental conditions. *Vet. Rec.* 147, 735-738
- 481
- 482 Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., De Kruif, A., 2001. Evaluation of the potential of
483 dogs, cats and rats to spread classical swine fever virus. *Vet. Rec.* 149, 212-213.
- 484
- 485 Donaldson, A.I., Alexandersen, S., 2002. Predicting the spread foot and mouth disease by airborne
486 virus. *Rev. sci. tech. Off. int. Epiz.* 21, 569-575.
- 487
- 488 Elbers, A.R., Stegeman, A., Moser, H., Ekker, H.M., Smak, J.A., Pluimers, F.H., 1999. The classical
489 swine fever epidemic 1997-1998 in the Netherlands: descriptive epidemiology. *Prev. Vet. Med.* 42,
490 157-184.
- 491
- 492 Elbers, A.R., Stegeman, J.A., de Jong, M.C., 2001. Factors associated with the introduction of
493 classical swine fever virus into pig herds in the central area of the 1997/98 epidemic in the
494 Netherlands. *Vet. Rec.* 149, 377-382.
- 495
- 496 Finney, D.J., 1978. *Statistical Methods in Biological Assay*, Charles Griffin & Company LTD, London,
497 pp. 394-401.
- 498
- 499 González, C., Pijoan, C., Ciprian, A., Correa, P., Mendoza, S., 2001. The effect of vaccination with the
500 PAV-250 strain classical swine fever (CSF) virus on the airborne transmission of CSF virus. *J. Vet.*
501 *Med. Sci.* 63, 991-996.
- 502
- 503 Hughes, R.W., Gustafson, D.P., 1960. Some factors that may influence hog cholera transmission. *Am.*
504 *J. Vet. Res.* 21, 464-471.

505

506 Kaden, V., Lange, E., Steyer, H., Bruer, W., Langner, C.H., 2003. Role of birds in transmission of
507 classical swine fever virus. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 50, 357-359.

508

509 Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., Deluyker, H., 1996.
510 Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density.
511 *Vet. Rec.* 139, 367-371.

512

513 Laevens, H., 1999. Risk factors for the transmission of classical swine fever virus to herds in the close
514 neighbourhood of an infected herd. In: *Epizootiology of classical swine fever: Experimental infections*
515 *simulating field conditions, and risk factors for virus transmission in the neighbourhood of an infected*
516 *herd. PhD thesis Ghent University, Belgium, pp 103-122.*

517

518 Laevens, H., Koenen, F., Deluyker, H., Berkvens, D., de Kruif, A., 1998. An experimental infection with
519 classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and
520 antibody response. *Vet. Q.* 20, 41-45.

521

522 Laevens, H., Koenen, F., Deluyker, H., de Kruif, A., 1999. Experimental infection of slaughter pigs with
523 classical swine fever virus: transmission of the virus, course of the disease and antibody response.
524 *Vet. Rec.* 145, 243-248.

525

526 Landman, W.J., van Eck, J.H., 2001. Aerosolization of Newcastle disease vaccine virus and
527 *Enterococcus faecalis*. *Avian Dis.* 45, 684-687.

528

529 Landman, W.J., Corbanie, E.A., Feberwee, A., Van Eck, J.H., 2004. Aerosolization of *Mycoplasma*
530 *synoviae* compared with *Mycoplasma gallisepticum* and *Enterococcus faecalis*. *Avian Pathol.* 33, 210-
531 215.

532

533 Liess, B., 1987. Pathogenesis and epidemiology of hog cholera. *Ann. Rech. Vet.* 18, 139-145.

534

- 535 May, K.R., 1966. Multistage liquid impinger. *Bacteriol. Rev.* 30, 559-570.
536
- 537 Ribbens, S., Dewulf, J., Koenen, F., Laevens, H., de Kruif, A., 2004. Transmission of classical swine
538 fever. A review. *Vet. Q.* 26, 146-155.
539
- 540 Schwarte, L.H., Mathews, J., 1954. Aerosol properties of lyophilized hog cholera virus. *Vet. Med.* 49,
541 233-234.
542
- 543 Sellers, R.F., Parker, J., 1969. Airborne excretion of foot-and-mouth disease virus. *J.Hyg. (Lond)* 67,
544 671-677.
545
- 546 Stärk, K.D., 1998. Failure to isolate classical swine fever virus from the air of rooms housing
547 experimentally infected pigs. In: *Systems for the prevention and control of infectious diseases in pigs.*
548 *PhD thesis Massey University. New Zealand, pp 171-176.*
549
- 550 Stärk, K.D., 1999. The role of infectious aerosols in disease transmission in pigs. *Vet. J.* 158, 164-181.
551
- 552 Stegeman, J.A., Elbers, A.R.W., Bouma, A., de Jong, M.C.M., 2002. Rate of inter-herd transmission of
553 classical swine fever virus by different types of contact during the 1997-8 epidemic in The
554 Netherlands. *Epidemiol. Infect.* 128, 285-291.
555
- 556 Stewart, W.C., Carbrey, E.A., Jenney, E.W., Kresse, J.I., Snyder, M.L., Wessman, S.J., 1975.
557 Transmission of hog cholera virus by mosquitoes. *Am J Vet Res* 36, 611-614.
558
- 559 Terpstra, C., 1987. Epizootiology of swine fever. *Vet. Q.* 9 Suppl 1, 50S-60S.
560
- 561 Van Oirschot, J.T., 1988. Description of the virus infection. In: Liess, B., *Classical Swine Fever and*
562 *Related Viral Infections, Martinus Nijhoff Publishing, Dordrecht, pp. 1-25.*
563

- 564 Van Rijn, P.A., Wellenberg, G.J., Hakze-van der Honing, R., Jacobs, L., Moonen, P.L., Feitsma, H.,
565 2004. Detection of economically important viruses in boar semen by quantitative RealTime PCR
566 technology. J. Virol. Methods. 120, 151-160.
- 567
- 568 Wensvoort, G., Terpstra, C., Boonstra, J., Bloemraad, M., Van Zaane, D., 1986. Production of
569 monoclonal antibodies against swine fever virus and their use in laboratory diagnosis. Vet. Microbiol.
570 12, 101-108.

571 **Tables**

572

573 Table 1. Effect of the gelatine filters on the viability of CSFV determined by virus titration. Stock

574 solutions (positive controls) were treated according to the same protocol as the filter solutions.

| Virus strain | Treatment | | Titer (TCID ₅₀ /ml) ^a | |
|--------------|---------------------|-------------------|---|----------------|
| | Volume ^b | Time ^c | Filter solution | Stock solution |
| Brescia | 50 ml | 30 min | 3.60 | 3.85 |
| | 50 ml | 5 min | 3.88 | 3.63 |
| | 5 ml | 5 min | 4.85 | 4.60 |
| Paderborn | 50 ml | 30 min | 3.35 | 3.60 |
| | 50 ml | 5 min | 3.50 | 3.75 |
| | 5 ml | 5 min | 4.85 | 4.60 |
| Zoelen | 50 ml | 30 min | 4.38 | 4.75 |
| | 50 ml | 5 min | 4.88 | 4.75 |
| | 5 ml | 5 min | 5.13 | 5.38 |

575 ^a) Based on control samples, always included in the virus titration, the standard deviation (SD) of the
576 virus titration technique is 0.43 TCID₅₀. This SD was calculated from 15 virus titration tests performed
577 in the lab in the course of this study.

578 ^b) Volume of medium used for dissolving the filter or stock.

579 ^c) Time interval between inoculation and processing of the filter or stock.

580

581 Table 2. Effect of the gelatine filters on the viability of CSFV determined by RRT-PCR. Stock solutions
 582 (positive controls) were treated according to the same protocol as the filter solutions.

| Virus strain | Treatment | | Cp value ^a | |
|--------------|---------------------|-------------------|------------------------------|-----------------------------|
| | Volume ^b | Time ^c | Filter solution ^d | Stock solution ^d |
| Brescia | 50 ml | 30 min | 29.8 | 30.2 |
| | 50 ml | 5 min | 30.1 | 29.8 |
| | 5 ml | 5 min | 26.3 | 27.1 |
| Paderborn | 50 ml | 30 min | 29.0 | 29.9 |
| | 50 ml | 5 min | 29.6 | 29.3 |
| | 5 ml | 5 min | 26.3 | 25.2 |
| Zoelen | 50 ml | 30 min | 25.9 | 25.5 |
| | 50 ml | 5 min | 25.2 | 25.6 |
| | 5 ml | 5 min | 21.9 | 22.2 |

583 ^a) Cp = crossing point; the standard deviation of the crossing points in the RRT-PCR has been
 584 calculated for the positive control samples and is 0.9, based on 9 RRT-PCR tests performed in the lab
 585 in the course of this study.

586 ^b) Volume of medium used for dissolving the filter or stock.

587 ^c) Time interval between inoculation and processing of the filter or stock.

588 ^d) A tenfold dilution of the samples were tested in the RRT-PCR.

589 Table 3. The aerosol concentration determined by virus titration of three different CSFV strains with time and their half-life time values. Air samples were
590 collected using the 2 min at 2 m³/h protocol.

591

| | Brescia | | | | Paderborn | | | | Zoelen | | | |
|---------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|
| | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | |
| | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) |
| Aerosol dose ^a | 6.43 | | 6.68 | | 6.18 | | 6.43 | | 7.93 | | 7.18 | |
| Control filter | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| Initial loss | 0.91 | | 1.66 | | 0.91 | | 0.41 | | 1.16 | | 0.41 | |
| t 0 | 5.52 | | 5.02 | | 5.27 | | 6.02 | | 6.77 | | 6.77 | |
| t 15 ^b | 4.52 | 4.5 | 5.02 | ∞ | 4.52 | 6.0 | 5.27 | 6.0 | 6.27 | 9.0 | 6.27 | 9.0 |
| t 30 ^b | 4.27 | 7.2 | 4.02 | 9.0 | 4.52 | 12 | 5.27 | 12 | 5.77 | 9.0 | 6.02 | 12 |

592 ^a) A slightly different aerosol dose was used in the duplicate experiments (experiment 1 and 2), however, the same aerosol doses were used for both sampling
593 protocols (Table 3 and 4).

594 ^b) The presented half-life time values at t 15 min and t 30 min were both calculated compared to t 0.

595 Table 4. The aerosol concentration determined by virus titration of three different CSFV strains with time and their half-life time values. Air samples were
 596 collected using the 10 min at 8 m³/h protocol.
 597

| | Brescia | | | | Paderborn | | | | Zoelen | | | |
|---------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|
| | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | | Experiment 1 | | Experiment 2 | |
| | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) | Log ₁₀ TCID ₅₀ / m ³ air | Half- life (min) |
| Aerosol dose ^a | 6.43 | | 6.68 | | 6.18 | | 6.43 | | 7.93 | | 7.18 | |
| Control filter | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| Initial loss | 0.61 | | 1.30 | | 0.15 | | 0.61 | | 1.15 | | 0.65 | |
| t 0 ^b | 5.82 | | 5.38 | | 6.03 | | 5.82 | | 6.78 | | 6.53 | |
| t 30 | 4.11 | 5.8 | 3.36 | 4.6 | 4.36 | 6.1 | 4.86 | 10 | 5.86 | 11 | 5.86 | 15 |

598 ^a) A slightly different aerosol dose was used in the duplicate experiments (experiment 1 and 2), however, the same aerosol doses were used for both sampling
 599 protocols (Table 3 and 4).

600 ^b) Due to the high relative humidity in combination with high air speed after aerosol generation, the filters taken at t 0 min clogged before the sampling time
 601 was completed. As in these cases less air was sampled, the results of the virus titration were corrected accordingly.

602 Table 5. VI, virus titration and RRT-PCR results of pigs infected with the Brescia strain

| Animal number | Virus isolation / titration ($\log_{10}\text{TCID}_{50}/\text{m}^3$) | | | | | | | | | RRT-PCR (Average Cp-value) | | | Calculated virus titration titers ^b ($\log_{10}\text{TCID}_{50}/\text{m}^3$) | | |
|---------------|--|---|---|------------|---|-----------------|------------------------|-----|---------------------|----------------------------|-------------------|---------------------|---|-----|---------------------|
| | Oropharyngeal swabs | | | Leucocytes | | | Samples of expired air | | Samples of cage air | Samples of expired air | | Samples of cage air | Samples of expired air | | Samples of cage air |
| | Dpi ^a : | 4 | 6 | 8 | 5 | 7 | 9 | 6 | 8 | 5 | 6 | 8 | 5 | 6 | 8 |
| 1 | + | + | + | + | + | nd ^a | - | - | - | Neg | 33.9 ³ | 36.1 ¹ | - | 2.2 | 0.3 |
| 2 | + | + | + | + | + | + | - | 3.5 | - | Neg | 29.9 ⁴ | 35.6 ² | - | 4.0 | 0.6 |
| 3 | + | + | + | + | + | + | - | - | - | Neg | 34.8 ¹ | Neg | - | 0.7 | - |
| 4 | + | + | + | + | + | + | - | - | - | Neg | 34.1 ³ | 34.0 ⁴ | - | 2.1 | 1.6 |
| 5 | + | + | + | + | + | + | - | - | - | Neg | 34.4 ³ | Neg | - | 2.1 | - |

603 ^a) nd = not done because of death; dpi = days post inoculation.604 ^b) Calculation based on the correlation between the virus titration assay and the RRT-PCR assay. The represented titers are the average of the four samples
605 tested in the RRT-PCR.606 ¹) one sample RRT-PCR positive out of four samples tested;607 ²) two samples RRT-PCR positive out of four samples tested;608 ³) three samples RRT-PCR positive out of four samples tested;609 ⁴) four samples RRT-PCR positive out of four samples tested.

610 Table 6. VI and RRT-PCR results of pigs infected with the Paderborn strain

| Animal number | Virus isolation | | | | | | | | | | | | RRT-PCR (Average Cp-value) | | | | | | Calculated virus titration titers ^b (log ₁₀ TCID ₅₀ /m ³) | | | | | | | | | | |
|---------------|---------------------|---|----|----|------------|---|----|----|------------------------|---|----|-----------------|----------------------------|---|----|------------------------|-------------------|-----|---|---------------------|-------------------|-------------------|----|-----|---|-----------------|-----|-----|-----|
| | Oropharyngeal swabs | | | | Leucocytes | | | | Samples of expired air | | | | Samples of cage air | | | Samples of expired air | | | | Samples of cage air | | | | | | | | | |
| | Dpi ^a : | 8 | 12 | 16 | 32 | 9 | 12 | 16 | 33 | 9 | 13 | 15 | 33 | 9 | 13 | 15 | 9 | 13 | 15 | 33 | 9 | 13 | 15 | | | | | | |
| 6 | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | Neg | Neg | Neg | 35.3 ³ | 33.8 ² | 36.2 ¹ | 33.9 ³ | - | - | - | 2.0 | 0.9 | 0.3 | 1.4 |
| 7 | + | + | + | - | + | + | + | - | - | - | - | nd ^a | - | - | - | Neg | Neg | Neg | nd ^a | Neg | Neg | Neg | - | - | - | nd ^a | - | - | - |
| 8 | + | + | + | - | + | + | - | - | - | - | - | nd ^a | - | - | - | Neg | Neg | Neg | nd ^a | Neg | 33.3 ¹ | Neg | - | - | - | nd ^a | - | 0.5 | - |
| 9 | + | + | + | - | + | + | + | - | - | - | - | nd ^a | - | - | - | Neg | Neg | Neg | nd ^a | Neg | Neg | Neg | - | - | - | nd ^a | - | - | - |
| 10 | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | Neg | 34.2 ¹ | Neg | 34.9 ³ | 35.7 ² | 33.8 ¹ | 34.2 ² | - | 0.4 | - | 2.2 | 0.6 | 0.5 | 0.9 |

611 ^a) nd = not done; dpi = days post inoculation.612 ^b) Calculation based on the correlation between the virus titration assay and the RRT-PCR assay. The represented titers are the average of the four samples
613 tested in the RRT-PCR.614 ¹) one sample RRT-PCR positive out of four samples tested;615 ²) two samples RRT-PCR positive out of four samples tested;616 ³) three samples RRT-PCR positive out of four samples tested;617 ⁴) four samples RRT-PCR positive out of four samples tested.

618 Table 7. VI and RRT-PCR results of pigs infected with the Zoelen strain

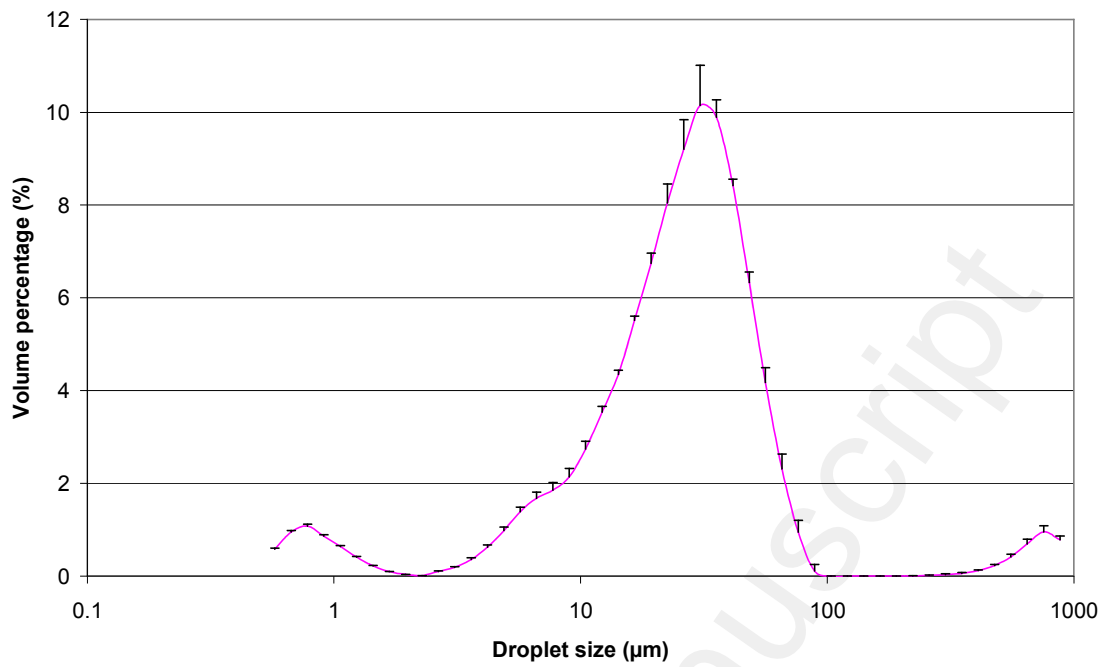
| Animal number | Virus isolation | | | | | | RRT-PCR | |
|---------------|----------------------|----|------------|----|------------------------|----|------------------------|-----|
| | Oropharyngeal swabs | | Leucocytes | | Samples of expired air | | Samples of expired air | |
| | Dpi ^a : 6 | 12 | 7 | 12 | 7 | 13 | 7 | 13 |
| 11 | + | - | + | - | - | - | Neg | Neg |
| 12 | + | - | + | - | - | - | Neg | Neg |
| 13 | + | - | + | - | - | - | Neg | Neg |
| 14 | + | - | + | - | - | - | Neg | Neg |
| 15 | + | + | + | + | - | - | Neg | Neg |

619 ^a) dpi = days post inoculation.

620

621 Figure 1. The average droplet size distribution with standard deviations of three aerosol spectra
622 generated by a Walther Pilot I spray-head.

Accepted Manuscript



ACCEPTED MANUSCRIPT