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Survival of classical swine fever virus at various temperatures in faeces and urine derived from experimentally infected pigs

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Abstract

Indirect transmission of classical swine fever virus (CSFV) can occur through contact with mechanical vectors, like clothing and footwear or transport vehicles, contaminated with the secretions or excretions of infected pigs. A prerequisite for indirect transmission is survival of the virus on the mechanical vector. Consequently, to obtain more insight into these transmission routes, it is important to know how long the virus remains viable outside the host. In this study we examined the survival of classical swine fever virus in faeces and urine derived from pigs intransally inoculated with a highly or moderately virulent CSFV strain. Faeces and urine were collected between days 5 and 36 post-inoculation, and stored at 5°C, 12°C, 20°C, and 30°C. Next, the virus titres were determined in the samples by virus titration, and a random selection of these samples was also analyzed by qRRT-PCR (Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction) to determine the viral RNA decay. Survival curves were generated, and it was shown that the inactivation rate was inversely related to the storage temperature. Average half-life values were between 2 and 4 days at 5°C, and between 1 and 3 hours at 30°C. Significant differences were observed in survival between virus strains in faeces, however, not in urine. The reduction in viral RNA during the entire study period was limited. This study provided detailed information on survival of CSFV in excretions of infected pigs, which can be used to improve control measures or risk analysis models.

Key words: Survival; Virus inactivation; Classical swine fever virus; Faeces and urine; Indirect transmission.

Introduction

Classical swine fever (CSF) is a highly contagious viral disease that caused several large outbreaks in the European Union in the last twenty years (Koenen et al., 1996; Elbers et al., 1999; Fritzemeier et al., 2000; Sharpe et al., 2001). One of the most disastrous examples is the 1997-1998 outbreak in Germany, The Netherlands, Belgium, Spain and Italy. In the Netherlands alone, approximately 11 million pigs were killed, mainly for welfare reasons (Terpstra and De Smit, 2000). During this outbreak different transmission routes contributed to the spread of the disease. Direct animal contact was in 17% of the cases responsible for transmission between herds before

implementing the first zoosanitary measures, including a total stand-still of transport of livestock (Elbers et al., 1999). Indirect transmission routes, however, played an important role in spread of the disease both before and after the implementation of movement restrictions (Elbers et al., 1999; Stegeman et al., 2002).

Indirect transmission can occur when susceptible pigs come into contact with mechanical vectors like clothing and footwear (Ribbens et al., 2007), or transport vehicles (Stegeman et al., 2002), contaminated with secretions or excretions of infected pigs. Saliva, nasal and lacrimal fluids, faeces, urine, and semen have been shown to contain significant amounts of CSFV (Weesendorp et al., 2008b; De Smit et al., 1999). A prerequisite for the indirect transmission is, however, the survival of the virus on the mechanical vector. Consequently, it is important to know how long the virus remains viable outside the host.

The survival of the virus on a vector depends on different variables like the initial amount of virus, temperature, pH, humidity, presence of organic matter, exposure to various chemicals (Edwards, 2000), and most likely other factors including properties of the strain (Depner et al., 1992). Conflicting results have been observed in survival times of CSFV in the environment. After spiking slurry (a mixture of faeces and urine, which may also contain (cleaning) water, small quantities of bedding material and feed) with CSFV, it survived for at least 70 days at 17°C, and for 84 days at 4°C (Eizenberger et al., reviewed by Haas et al., 1995). However, Bøtner reported no detectable amounts of infectious virus in slurry stored at 20°C after 2 weeks (reviewed by Haas et al., 1995). Pens contaminated with secretions and excretions of infected pigs, contained infectious virus for at least ten hours when the environmental temperature was around 22°C (Ribbens et al., 2004). Depending on the temperature, contaminated pens probably contain infectious virus for a few days before the virus within the pen and manure is totally inactivated (Artois et al., 2002). In certain housing systems, where temperatures decrease during winter conditions, the survival time might be prolonged, and pens may contain virus in excreta and bedding for at least four weeks (Harkness, 1985).

These studies give useful information on survival times of CSFV in the environment, but details and conditions of the performed experiments were limited reported, or presented survival times were not specific, or only based on spiking of slurry. None of the studies on survival of CSFV in the environment contaminated with excretions or secretions provided sufficient

information for the construction of survival curves. Concerning the importance of indirect transmission routes during outbreaks, specific information on virus survival is necessary (Edwards, 2000; De Vos et al., 2006). This can be used to model the risk of transmission via different transmission routes, which supports improvement of control measures.

In this report the survival of CSFV in faeces and urine from infected pigs was studied, as these excretions are produced in large amounts and are major sources of contaminating the immediate surroundings and mechanical vectors.

Materials and Methods

Experimental animals and housing

Two experiments were performed in succession with eight weeks old male pigs, obtained from a conventional, but pestivirus free pig herd in the Netherlands. Pigs were individually housed in cages to collect faeces and urine. Pigs were fed once a day with commercial feed for finishing pigs, and water was provided ad libitum.

Viruses and inoculation of animals

Five pigs were inoculated intranasally with a dose of 100 LD_{50} (50% lethal dose), which is approximately $10^{2.5}$ $TCID_{50}$ (50% tissue culture infectious dose) of the highly virulent Brescia strain (genotype 1.2, strain Brescia 456610, obtained from Brescia, Italy, 1951 (Wensvoort et al., 1989)). Seven pigs were inoculated with 10^5 $TCID_{50}$ of the moderately virulent Paderborn strain (genotype 2.1, isolated in 1997 during the outbreak in the Paderborn area of Germany (Greiser-Wilke et al., 2000)). One ml of the virus suspension was administered per animal, 0.5 ml per nostril.

Sampling and pre-treatment of samples

Faeces and urine samples were collected from pigs infected with the Brescia strain from day 5 post-inoculation (p.i.), and from pigs inoculated with the Paderborn strain from day 10 p.i. The Brescia strain was previously isolated from faeces at day 6 p.i. (Ressang, 1973). The Paderborn strain was expected to be present in faeces and urine later, therefore no samples were collected before day 10 p.i. Samples were collected until day 12 p.i. from Brescia-infected animals (when the last one died) and until day 36 p.i. from Paderborn infected animals, as long as they showed

clinical symptoms. Samples that were negative from the start of the inactivation period were excluded from the analysis. Faeces were obtained from the rectum by stimulation of the anus, and urine was collected when pigs urinated. Directly after collection the samples were stored in a refrigerator at 5°C to avoid inactivation of the virus until samples were transported to the laboratory.

In the laboratory, samples were directly stored in 50 ml tubes in a refrigerator at 5°C, a thermostated water bath at 12°C, a thermostated room at 20°C, or a thermostated water bath at 30°C. Individual samples were used if enough faeces or urine could be obtained from one pig. In case not enough faeces or urine was available from one pig, samples originating from pigs infected with the same strain and on the same day p.i. were pooled. Samples were thoroughly mixed before storage. Depending on the temperature, samples were taken from the stored faeces and urine at different time intervals. These sampling moments were determined after a pilot-study in which the rate of inactivation was studied on a small amount of samples. As the pilot study showed that inactivation of virus occurs more rapidly in urine than in faeces at lower temperatures, urine samples were stored for a shorter duration at 5°C and 12°C than faeces samples. Faeces samples were stored for maximum 45 days at 5°C, 41 days at 12°C, and 5 days at 20°C or 30°C. Urine samples were stored for maximum 27 days at 5°C, 15 days at 12°C, and 5 days at 20°C or 30°C. The number of samples per strain-temperature combination that was ultimately tested, ranged from 5 to 10, depending on availability of faeces or urine, presence of virus in the initial sample, and (for Paderborn) inclusion of the results from the pilot study.

Faeces samples were diluted 1:10 in medium (Eagle minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and 10% antibiotics solution ABII (1000 U/ml Penicillin, 1 mg/ml Streptomycin, 20 µg/ml Fungizone, 500 µg/ml; Polymixin B, and 10 mg/ml Kanamycin)) and vortexed with glass beads. After centrifugation (10.000 ***g*** for 5 minutes) the supernatants were stored at -70°C until analysis. From urine a 1:10 dilution in medium (EMEM containing 10% FBS and 10% antibiotics) was prepared and stored at -70°C until analysis. All samples were analyzed by virus titration. Four randomly selected samples from both faeces and urine, and from each virus strain and each temperature, were analyzed by quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRRT-PCR). Furthermore, the pH of two urine samples from each storage temperature were determined at each sampling moment.

144

145 *Virus titration*

146 Virus titration was performed as described by Weesendorp et al. (2008a). Briefly, a volume of 250
147 µl of the sample was incubated for one hour on a monolayer of SK6 cells in a 24-wells plate
148 (Greiner). After a wash procedure with PBS (phosphate-buffered saline), EMEM supplemented
149 with 5% FBS and 10% antibiotics, was added to the wells. The plates were incubated at 37°C in
150 an atmosphere with 5% CO_2 for four days. After being fixated and washed, the monolayers were
151 stained by the immuno-peroxidase technique (Wensvoort et al., 1986) using two HRPO-
152 conjugated CSFV specific MAbs (V3/V4) and examined for stained cells. Virus titres were
153 calculated as $TCID_{50}$ using the Spearman-Kärber method (Finney, 1978). The detection limit with
154 this method is $10^{1.1}$ $TCID_{50}$/ml of urine or gram faeces.

155

156 *Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRRT-PCR)*

157 The concentration of viral RNA in the excretions was analyzed by qRRT-PCR. For RNA isolation
158 200 µl of the sample was pipetted manually into MagNA Pure sample cartridges (Roche Applied
159 Science, Mannheim, Germany). In each run of thirty-two samples two negative control samples
160 and five dilutions of a positive control sample (standard curve) were included. The standard
161 curves were constructed by spiking faeces and urine with known concentrations of infectious virus
162 from the Brescia or Paderborn strain. The RNA was extracted with the Total Nucleic Acid Isolation
163 Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions
164 using the automated MagNA Pure LC instrument (Roche Applied Science, Mannheim, Germany).
165 After RNA isolation, the nucleic acids were immediately processed for the qRRT-PCR or stored at
166 -70 °C in the sample cartridge until the PCR was carried out.

167 The qRRT-PCR was performed with a LightCycler (LC) instrument (Roche Applied
168 Science, Mannheim, Germany) using the RNA Master Hybridization Probes Kit, as described by
169 Van Rijn et al. (2004). Analysis was performed with the LC software. The viral RNA concentration
170 ($TCID_{50}$ equivalents per ml or gram) of each individual sample could be calculated using the
171 standard curve. The standard curves were constructed based on Cp (crossing point) values for all
172 dilutions of the positive control. The Cp value is the cycle number at which the fluorescence

emission from a PCR rises above the background signal. A low Cp value indicated high template amount, while a high Cp indicated a low template amount.

Statistical analysis

Per sample, the half-life (*h)* of CSFV in faeces or urine was estimated by:

$$h = -\log_{10} 2 / b$$

where *b* is the least squares estimate of the slope of the regression of $\log_{10}$ CSFV over time for that sample. To study the effect of temperature on virus survival, the dependence of the log transformed half-life value on temperature was modelled. Half-life values were log transformed in order to ensure homogeneity of variance in the regression models that were used. The log-transformed half-life ln(*h*) was analysed as a new derived response variable with a linear mixed model (Searle et al., 1992). The model comprised random effects, to model correlation between samples from a common origin, main effects for strains, linear and quadratic effects for temperature and interaction between strains and temperature. Components of variance were estimated by restricted maximum likelihood (REML). Significance tests for interaction, slopes of linear and quadratic terms in time and main effects for strains were based on the Wald test (Cox and Hinkley, 1974). All calculations were performed with the statistical programming language GenStat (2007).

Results

Inactivation of infectious virus in faeces and urine derived from infected pigs

The mean half-life values for CSFV in faeces and urine derived from pigs infected with the highly virulent Brescia or moderately virulent Paderborn strain are shown in Tables 1 and 2. The initial titres of infectious virus in faeces of pigs infected with the Brescia strain were $10^{2.6}$ to $10^{5.4}$ $TCID_{50}$/g, and of pigs infected with the Paderborn strain $10^{3.6}$ to $10^{5.4}$ $TCID_{50}$/g. Initial virus titres in urine of pigs infected with the Brescia strain were $10^{3.4}$ to $10^{5.9}$ $TCID_{50}$/ml, and in urine of pigs infected with the Paderborn strain $10^{1.9}$ to $10^{6.9}$ $TCID_{50}$/ml.

The pH of the urine samples remained 7 for the duration of the experiment. However, the urine became darker during the experiment and a granular precipitate developed on the bottom of the tube.

Effect of temperature on the half-life values of the virus

The regression model comprised both linear and quadratic effects of temperature and interaction between strains and temperature. For faeces samples the quadratic effect and interaction between strains and temperature were not significant and subsequently omitted from the model, resulting in the linear relationship in Figure 1A. The Wald test showed for urine samples that the interaction between strains and temperature were not significant, but linear and quadratic effects were significant (p = 0.001), which explains the parabolic shape in Figure 2A.

The analysis showed a significant difference (common slope but different intercepts on the logarithmic scale: p = 0.03) between the Brescia and Paderborn strain for half-life in relation to temperature in faeces, but not in urine. For that reason, curves with a separate intercept per strain were fitted and shown in Figure 1A, while a single common curve was fitted and shown in Figure 2A.

In Figures 1B (faeces) and 2B (urine) the direct relationship between half-life (untransformed) and temperature is shown. Also shown in Figures 1A, 1B, 2A and 2B are the 95% confidence intervals around the fitted curves and the actual data. Figures 1B and 2B clearly show that variation among the data is larger for lower temperatures, while variation is reasonably constant for all temperatures after log transformation in Figures 1A and 2A.

Estimated survival of classical swine fever virus in faeces and urine

The presented days until inactivation of the virus (to a level below the detection limit of the virus titration assay) are calculated using the models presented in Figures 1 and 2.

Furthermore, the estimated survival of CSFV in faeces and urine (Table 3) are based on the maximum amounts of virus detected in these excretions from infected pigs (Weesendorp et al., 2008b). In faeces from pigs infected with the Brescia strain, the maximum amount of virus detected was $10^{5.6}$ $TCID_{50}$/g (day 8 p.i.), and in faeces from pigs infected with the Paderborn strain $10^{6.1}$ $TCID_{50}$/g (day 38 p.i.). In urine from pigs infected with the Brescia strain a maximum of $10^{5.9}$ $TCID_{50}$/ml (day 9 p.i.) was detected, and in urine from pigs infected with the Paderborn strain $10^{6.9}$ $TCID_{50}$/ml (day 32 p.i.).

Viral RNA concentration over time in faeces and urine after storage at different temperatures.

The reduction of viral RNA in faeces over time was limited (Figure 3). The maximum decrease of viral RNA concentrations were $10^{1.7}$ $TCID_{50}$ equivalents at 5°C, $10^{1.0}$ $TCID_{50}$ equivalents at 12°C, $10^{1.3}$ $TCID_{50}$ equivalents at 20°C, and $10^{2.1}$ $TCID_{50}$ equivalents at 30°C. In urine the viral RNA concentrations remained quite stable, although at 30°C in all samples a reduction (> $10^{0.7}$ $TCID_{50}$ equivalents) of the viral RNA concentrations were observed (Figure 4). The maximum decrease of viral RNA concentrations were $10^{1.1}$ $TCID_{50}$ equivalents at 5°C, $10^{1.0}$ $TCID_{50}$ equivalents at 12°C, $10^{1.4}$ $TCID_{50}$ equivalents at 20°C, and $10^{2.8}$ $TCID_{50}$ equivalents at 30°C.

Discussion

A prerequisite for indirect transmission is virus survival in the environment. Until now only limited information on virus survival was available. Furthermore, most studies focused on virus survival in medium or slurry (Depner et al., 1992; Haas et al., 1995). For indirect transmission via persons or transportation trucks, however, faeces and urine are more relevant than slurry. The present study filled these gaps by generating survival curves of CSFV in faeces and urine derived from pigs infected with a highly or moderately virulent CSFV strain.

The model presented in this study made it possible to estimate survival times of CSFV in faeces and urine for different environmental temperatures. In Table 3 results are presented of survival times when maximum amounts of virus are excreted by infected pigs (Weesendorp, 2008b). Survival times of this worst-case scenario are presented with 95% confidence intervals, based on the confidence intervals of the model (Figures 1 and 2). These confidence intervals are relatively wide, due to the large variation between samples (especially at low temperatures), and the small number of samples. As this worst-case scenario is calculated using maximum amounts of virus, survival times at low temperatures are in some cases longer than the study period of the samples, which likely resulted in an overestimation of the maximum survival time.

Within the studied temperature range, there is an inverse relationship between virus survival and temperature, which other studies already demonstrated. In spiked slurry with an initial concentration of $10^{5.5}$ $TCID_{50}$/ml the virus was not inactivated at 5°C within 42 days (Bøtner, reviewed by Haas et al., 1995). Based on this initial concentration of $10^{5.5}$ $TCID_{50}$/ml and the model presented in this study, virus in faeces produced by infected pigs would on average be

undetectable within 42 (Paderborn) or 64 days (Brescia). In urine produced by infected pigs, the virus would be inactivated to a level below the detection limit within 18 days (Paderborn and Brescia). These results would suggest that virus survival in faeces is comparable to that in slurry (Bøtner, reviewed by Haas et al*.,* 1995). However, virus survival in slurry kept at other temperatures is different in the study of Bøtner than virus survival in faeces in the present study. Bøtner (reviewed by Haas et al*.,* 1995) reported an inactivation time of 14 days for virus in slurry kept at 20°C, while in the present study the virus would be inactivated in faeces at this temperature within 3 (Paderborn) or 5 (Brescia) days. These differences could be due to differences in condition between slurry and faeces or urine, differences in storage condition, spiking versus using faeces and urine from infected pigs, differences between the methods used for isolation of the virus, and differences between the detection assays.

Studies on survival of virus in excretions of infected pigs are rare. Pens contaminated with secretions and excretions of infected pigs showing clinical symptoms, contained infectious virus for at least ten hours when the environmental temperature was around 22°C (Ribbens et al., 2004). Given equal circumstances, the duration of survival will be mainly dependent on the initial concentration of virus in the pen. Pigs infected with highly virulent or moderately virulent strains of CSFV can excrete large quantities of virus when clinical signs are present. Via the faeces a maximum of $10^{9.9}$ $TCID_{50}$/Paderborn-infected pig or $10^{8.4}$ $TCID_{50}$/Brescia-infected pig was produced per day. Via the urine a maximum of $10^{9.0}$ $TCID_{50}$/Paderborn-infected pig or $10^{7.8}$ $TCID_{50}$/Brescia-infected pig was produced per day (Weesendorp et al, 2008b). Based on the model presented here, it can be calculated that virus produced in one of these days would survive for 6 (Brescia) or 4 days (Paderborn) in faeces, and 2 (Brescia) or 3 (Paderborn) days in urine at 22°C. This agrees with previous observations that pens housing infected pigs, contain infectious virus for a few days before the virus within the pen and manure is totally inactivated (Artois et al., 2002).

The interest in the present study is not only to predict virus survival, but also its relationship to the transmission of disease to healthy pigs. To determine CSFV survival, we used the detection limit of the cell culture assay (virus titration). Information on the relationship between the detection limit of the assay and the sensitivity of the animal to infection is, however, scarce. In the present study the detection limit of the virus titration assay was 13 $TCID_{50}$/g faeces or ml of

293 urine. The minimal infective dose that results in fatal disease after inoculation with the highly
294 virulent strain "Alfort" is 10 $TCID_{50}$ per pig (Liess, 1987). For the Brescia virus strain a pig ID_{50} of
295 80 $TCID_{50}$ was determined after intranasal inoculation of virus in medium (Terpstra and
296 Wensvoort, 1988). Thus, it seems that the detection limit of the virus titration gives a fair prediction
297 of the transmission to healthy pigs. However, in previous studies the host animal has proven to be
298 more sensitive than tissue culture (Stewart et al., 1979; McKercher et al., 1987; Panina et al,
299 1992), although it must be considered that the sample amounts tested in *in vitro* assays are
300 mostly much smaller than in the *in vivo* assays. Furthermore, the minimum infective intranasal
301 dose of virus in medium might be different from minimum infective oral doses of virus in faeces or
302 urine. It is therefore difficult to predict whether the risk of infection is over-or underestimated.
303 Indirect transmission routes play a major role in transmission of the virus. The importance
304 of contaminated livestock trucks in introduction of the virus into the Netherlands has been shown
305 by De Vos et al. (2004). Their model indicated that returning livestock trucks contributed most
306 (about 65%) to the probability of CSFV introduction in the Netherlands. However, many
307 intermediate steps are involved in this transmission route: the livestock truck visiting an infected
308 farm is in contact with infected pigs or infectious material (even though pigs showing clinical
309 symptoms are not allowed to be transported), the livestock truck gets contaminated, the virus
310 survives during transportation, the virus is not removed or inactivated by cleaning and disinfecting,
311 there is contact between the livestock truck and susceptible pigs, and an infective viral dose is
312 transmitted. In spite of the small probability of all these events occurring, such worst-case
313 scenarios have happened in the past. The 1997-1998 outbreak is supposed to be caused by a
314 transportation truck returning from the Paderborn region in Germany. Due to the low temperatures
315 (-10°C to -20°C) the cleaning and disinfection of the truck was hampered, and the virus survived
316 (Elbers et al., 1999). The present study showed that even at higher temperatures the virus can
317 survive for a sufficient period to be transported over a long distance. Based on initial
318 concentrations used in Table 3 ($10^{5.6}$ to $10^{6.1}$ $TCID_{50}$), CSFV can survive for 4 to 5 days in faeces
319 at 20°C, and 15 to 20 hours in faeces at 30°C.
320 Differences in survival were observed between the virus strains. The Brescia strain was
321 more resistant to inactivation in faeces than the Paderborn strain. This was also observed in urine,
322 although these differences were small, and not significant. Differences between strains were

observed before in a survival study with three cell culture-propagated strains of CSFV exposed to various temperatures and hydrogen ion concentrations (pH)(Depner et al., 1992). The Brescia strain showed the highest half-life values at 4°C or 21°C and pH4. At lower hydrogen ion concentrations the 331/USA or Osterrode 2699/83 strains showed higher half-life values. The differences between virus strains in survival might be due to differences in the properties of the virus particle (e.g. the protein capsid and envelope proteins) or differences in the level of cell-association. Furthermore, faeces from pigs infected with the highly virulent Brescia strain could contain higher amounts of blood, as more clinical signs and haemorrhages were observed than of pigs infected with the Paderborn strain. This higher amount of blood in the faeces could have resulted in a more favourable environment for the virus.

The viral RNA level remained rather stable during the entire study period. The viral RNA reduction was in most cases within the variation of the qRRT-PCR assay. Therefore, it was not possible to calculate reliable half-life values for viral RNA. To do so, samples should be studied for a longer period. It is clear that stability of viral RNA does not predict the presence of infectious CSFV. However detection of CSFV-RNA in faeces and urine might be important from a diagnostic point of view. It will be possible to demonstrate the virus originally being present, even after a long period when samples are stored at higher temperatures.

Spiking has been used primarily to study survival of viruses (Bøtner, 1991; Haas et al., 1995; Turner et al., 2000). Based on the results of the spiking studies restrictions have been implemented after an outbreak to prevent recrudescence of the disease, like long-time storage of slurry (42 days in the European Union (Anonymous, 2001)), treatment of slurry, or decontamination of mechanical vectors like livestock trucks, boots or clothing. So far, however, it is not known whether spiking is an appropriate method to study survival in excretions produced by infected pigs. Especially with a hemorrhagic disease as CSF, faeces and urine can contain blood or infected cells, which might increase the survival time. Therefore, the approach used in the present study, using faeces and urine from infected animals, is a more realistic estimate of the behaviour of CSFV survival in excretions.

The decision whether or not to implement certain control measures, based on risk-analysis models, depends heavily on the reliability of available data. For the model described by De Vos et al. (2004), only limited data were available to estimate the probability of CSFV survival

in an empty livestock truck travelling over a distance to 900 km. It was suggested that studies should be performed to estimate this parameter more precisely (De Vos et al., 2006). These data have been generated in the present study.

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488 Table 1. Survival of CSFV at different temperatures in faeces derived from pigs infected with the

489 Brescia or Paderborn strain

Temperature(°C)	Virus strain	Mean half-life (days)	SEM[1]	Min[2]	Max[3]	*n*[4]
5	Brescia	3.50	0.58	2.41	5.69	5
	Paderborn	2.90	0.58	1.38	5.66	8
12	Brescia	2.69	0.81	0.99	6.40	6
	Paderborn	1.67	0.29	0.47	2.76	8
20	Brescia	0.28	0.05	0.14	0.42	6
	Paderborn	0.14	0.04	0.03	0.30	7
30	Brescia	0.06	0.01	0.04	0.09	6
	Paderborn	0.07	0.02	0.02	0.14	7

490

491 [1] SEM = standard error of the mean.

492 [2] Min = minimum half-life value observed.

493 [3] Max = maximum half-life value observed.

494 [4] *n* = number of samples tested.

Table 2. Survival of CSFV at different temperatures in urine derived from pigs infected with the Brescia or Paderborn strain

Temperature(°C)	Virus strain	Mean half-life (days)	SEM[1]	Min[2]	Max[3]	*n*[4]
5	Brescia	2.82	2.25	0.39	11.81	5
	Paderborn	2.14	0.79	0.33	6.56	9
12	Brescia	0.54	0.25	0.18	1.54	5
	Paderborn	0.41	0.13	0.02	1.39	10
20	Brescia	0.19	0.11	0.06	0.61	5
	Paderborn	0.21	0.15	0.03	0.12	6
30	Brescia	0.12	0.08	0.03	0.41	5
	Paderborn	0.08	0.04	0.03	0.25	5

[1] SEM = standard error of the mean.

[2] Min = minimum half-life value observed.

[3] Max = maximum half-life value observed.

[4] *n* = number of samples tested.

503 Table 3. Estimates of the duration of survival of CSFV in faeces and urine when maximum
504 amounts of virus are present, at the peak of virus excretion [a]

Temperature(°C)	Virus strain	Mean survival in faeces (days)[b]	95% confidence interval	Mean survival in urine (days)[c]	95% confidence interval
5	Brescia	66	(16-272)	20	(1.7-243)
	Paderborn	47	(11-194)	23	(1.9-283)
12	Brescia	19	(4.7-80)	4.9	(0.41-59)
	Paderborn	14	(3.4-57)	5.6	(0.46-68)
20	Brescia	4.8	(1.2-20)	1.8	(0.15-22)
	Paderborn	3.5	(0.84-14)	2.1	(0.17-25)
30	Brescia	0.85	(0.20-3.5)	1.4	(0.12-17)
	Paderborn	0.61	(0.15-2.5)	1.6	(0.13-20)

505

506 [a] Mean survival is based on models presented in Figures 1 and 2. Survival is defined as duration
507 of virus infectivity until virus titres were below the detection limit of the virus titration assay ($10^{1.1}$
508 $TCID_{50}$/ g or ml).

509 [b] Calculations based on maximum excretion of CSFV in faeces of pigs infected with the Brescia
510 strain: $10^{5.6}$ $TCID_{50}$/g , of pigs infected with the Paderborn strain: $10^{6.1}$ $TCID_{50}$/g.

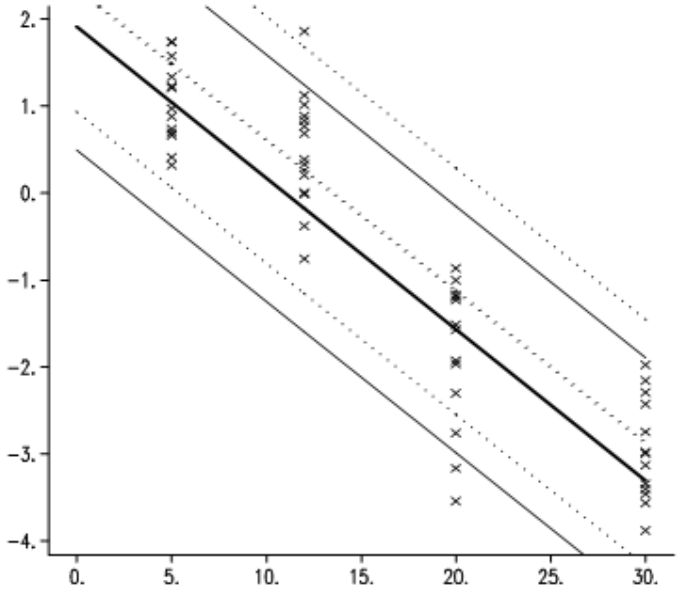
511 [c] Calculations based on maximum excretion of CSFV in urine of pigs infected with the Brescia
512 strain: $10^{5.9}$ $TCID_{50}$/ml , of pigs infected with the Paderborn strain: $10^{6.9}$ $TCID_{50}$/ml.

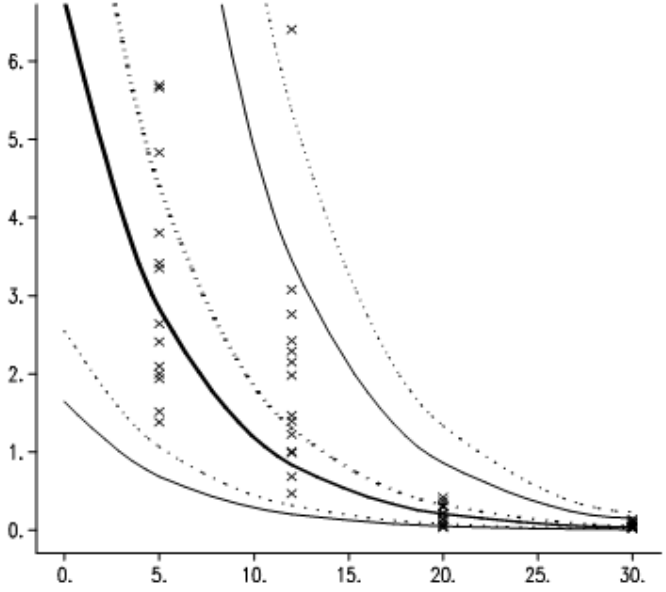
Figure 1. Effect of faeces temperature on virus survival. (A) effect of faeces temperature (x-axis) on the log transformed half-life values (y-axis) of the virus. (B) effect of faeces temperature on the half-life values of the virus. The relation between temperature and survival is presented with 95% confidence intervals for both the (**....**) Brescia and (—) Paderborn strain of CSFV. Also shown are the half-life values of the individual faeces samples (x).

Figure 2. Effect of urine temperature on virus survival. (A) effect of urine temperature (x-axis) on the log transformed half-life values (y-axis) of the virus. (B) effect of urine temperature on the half-life values of the virus. The relation between temperature and survival is presented with 95% confidence intervals for both the (**....**) Brescia and (—) Paderborn strain of CSFV. Also shown are the half-life values of the individual urine samples (x).

Figure 3. Viral RNA concentration over time in faeces samples from pigs infected with the Brescia or the Paderborn strain. Samples were stored at different temperatures.

Figure 4. Viral RNA concentration over time in urine samples from pigs infected with the Brescia or the Paderborn strain. Samples were stored at different temperatures.

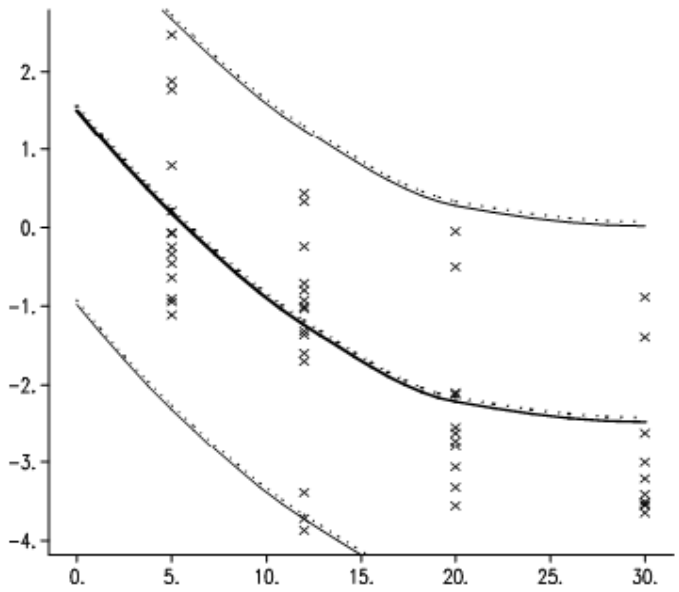


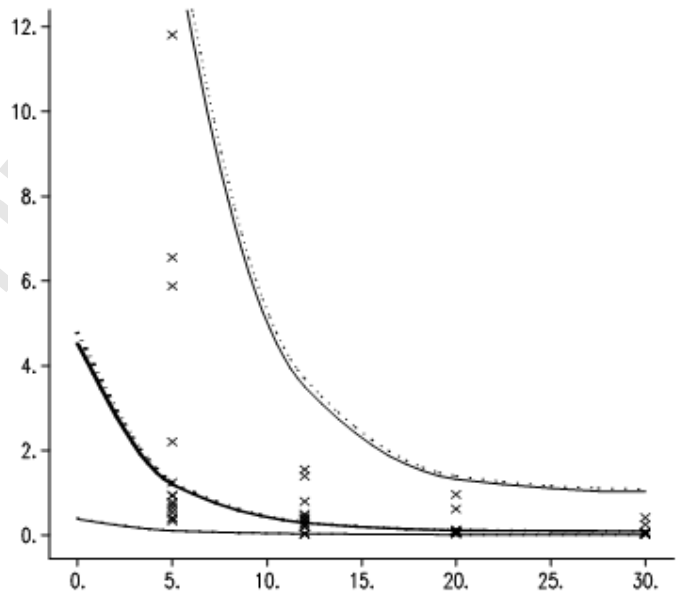


A Brescia : Log(Half-life) = 2.353 – 0.1741*temp
Paderborn : Log(Half-life) = 1.913 – 0.1741*temp

B Brescia : Half-life = $e^{2.353- 0.1741*temp}$
Paderborn : Half-life = $e^{1.913- 0.1741*temp}$

Figure 1.





A Brescia : $\text{Log(Half-life)} = 1.562 - 0.2928*\text{temp} + 0.005337*\text{temp}^2$
Paderborn : $\text{Log(Half-life)} = 1.508 - 0.2928*\text{temp} + 0.005337*\text{temp}^2$

B Brescia : $\text{Half-life} = e^{1.562 - 0.2928*\text{temp} + 0.005337*\text{temp2}}$
Paderborn: $\text{Half-life} = e^{1.508 - 0.2928*\text{temp} + 0.005337*\text{temp2}}$

Figure 2.

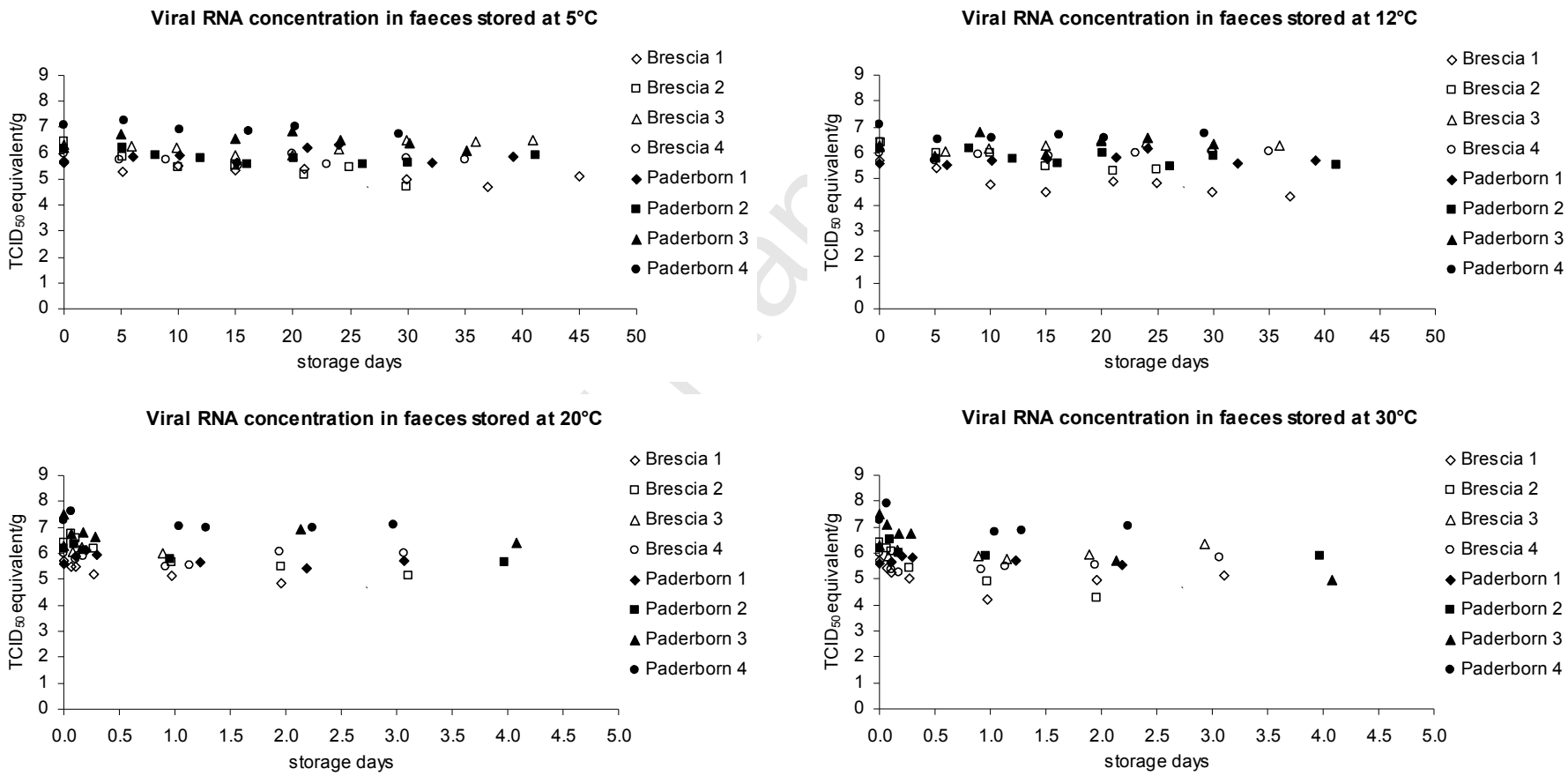


Figure 3.

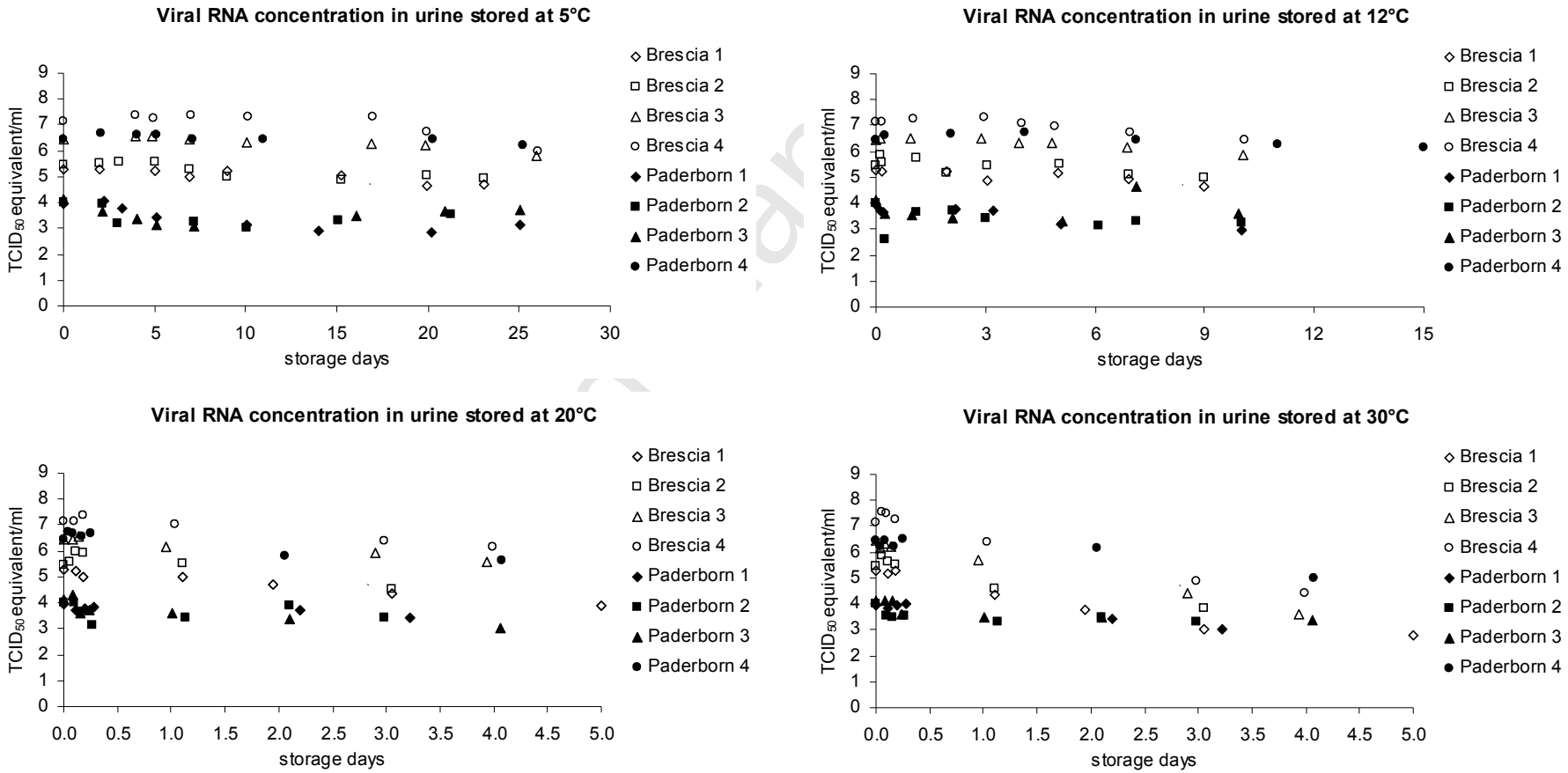


Figure 4.