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Virulence of Classical Swine Fever Virus Isolates from Europe and other areas during 1996 until 2007

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Abstract

Classical Swine Fever (CSF) has caused several outbreaks in EU Member States with grave economic consequences. Several times the diagnosis of CSF was made too late partially due to non-specific clinical signs which did not raise suspicion for CSF. Virulence of CSF virus isolates (CSFV) still remains a subject of discussion and speculation as sufficient knowledge is still not available. Six uncharacterised CSFV isolates from 1996 until 2007 were assessed in animal experiments for their clinical virulence in order to broaden the knowledge about circulating CSFV and thereby assist disease eradication. A clinical (CS) and pathological score were applied and further extended by additional parameters to a modified CS (mCS) including case fatality, antibody production and leukocyte count.

The unknown CSFV isolates could be classified as moderately or highly virulent. The inclusion of additional parameters, especially case fatality, into the mCS gave a more reliable classification of virulence, proving that there are clinical signs and laboratory parameters of blood which can be recognised. Therefore a subclinical course of infection is unlikely, especially in weaner pigs.

Keywords

Classical Swine Fever – virulence – clinical score – pathological lesions – differential diagnosis – case fatality.

Introduction

Classical Swine Fever (CSF), which is among the diseases notifiable to the World Organisation for Animal Health (OIE) (Anonymous, 2004), occasionally causes sporadic epidemics in EU Member States and is endemic in a number of Third Countries worldwide. Since CSF has been generally eradicated from the EU, the control measures for CSF are based on a non-vaccination policy (Anonymous, 2001). However, during the last decades several

reintroductions of CSF virus (CSFV) have caused epidemics of severe economic consequences (Elbers et al., 1999; Fritzemeier et al., 2000; Sandvik et al., 2000). Whether there has been a change in virulence of the virus over time is a constant subject of discussion. CSFV isolates isolated from EU Member States from 1997 and 2001 have been classified as moderately virulent (Floegel-Niesmann et al., 2003). With these strains, clinical signs may be rather non-specific and age-dependent. This made diagnosis difficult and a new CSF outbreak was often discovered too late. Textbook cases with haemorrhagic lesions like bleedings of skin, petechiae on kidney and tonsils as well as spleen infarctions (Mengling and Packer, 1969) were not frequently observed. Only lymphadenosis, and high body temperature were common features of CSF infected pigs, but for clinical and pathological diagnosis this is rather non-specific (Floegel-Niesmann et al., 2003).

Characterising virulence has been attempted in various ways: case fatality, clinical and pathological signs (Carbrey et al., 1980, Wood et al. 1988), observations in CSF infected animal farms (Elbers et al., 2002), characterization of CSFV in cell cultures (Kubin 1967; Mittelholzer et al., 2000) or differences in the genome (Moormann et al., 1996; Mayer et al., 2003). Most of these characterisations are restricted to individual strains and they do not apply for other CSFV strains. Whether characterisation of virulence performed about three decades ago, still applies to CSFV isolated since the vaccination policy in the EU stopped at the beginning of the nineties is therefore questionable.

Mittelholzer et al. (2000) started to define objective criteria for the evaluation of clinical signs using a clinical score. Floegel-Niesmann et al. (2003) extended this score by pathological signs to allow for a better discrimination and thus comparison.

The purpose of this study was to characterise six so far unknown CSFV, isolated in Russia, Guatemala, South Africa, the Balkan area and the most recent EU Member State Bulgaria, using an extended clinical score and an established pathological score in order to increase the

knowledge on virulence of CSFV which are still circulating in the pig population in different parts of the world.

Material and methods

Five CSFV isolated in Third Countries and one CSFV from Bulgaria were compared with two formerly characterised CSFV from EU Member States. CSF0695 was isolated in Russia from a domestic pig in 1996 and thus represents a CSFV circulating in Russia different from those circulating in the EU (Vlassova et al., 2003). CSF0650 was isolated from a domestic pig from Guatemala in 1999 and represents CSFV from Central America and The Caribbean (Pereda et al., 2005). CSF 0695 belongs to genetic subtype 1.1 whereas CSF0650 belongs to genetic subtype 1.3 (Greiser-Wilke et al., 2006). CSF0849 was isolated from domestic pigs in South Africa in 2005 (Sandvik et al., 2005). CSF0854 was isolated from domestic pigs in the Republic of Kosovo in 2006 and CSFV0870 was isolated from domestic pigs in 2007 in Croatia. CSF0864 was isolated in Bulgaria from domestic pigs in 2007. The genetic typing revealed that CSF0854, CSF0864, and CSF0870 belonged to genotype 2.3, and CSF0849 belonged to genotype 2.1. Both genotypes were also isolated in EU Member States during the last decade (Greiser-Wilke et al., 2006).

Two CSFV from EU Member States (CSF0277 and CSF0634) have been characterised previously as moderately virulent (Floegel-Niesmann et al., 2003) and were used for comparison. CSF0277 (genetic subtype 2.1) caused the CSF epidemic in domestic pigs in 1997 affecting several EU Member States. CSF0634 (genetic subtype 2.3) was isolated from a CSF outbreak in domestic pigs in 2001 in Germany and was also present in the local wild boar population for several years (Fritzemeier et al., 2000) .

The CSFV were cultivated on PK 15(A) cells and their virus titre determined prior to inoculation of the pigs (Anonymous, 2002). The CSF antibody titres against the homologue

CSFV were obtained by neutralisation test (Anonymous, 2002). Leukocyte counts on EDTA blood samples were performed according to standard haematological procedure.

Animal experiments

Among the duties of the EU Reference Laboratory for CSF are the characterisation of CSFV isolates from new CSF outbreaks and the production of reference material for laboratory diagnosis (Anonymous 2001). In this framework, experiments were conducted according to the German Animal Welfare Act. Serum and organ materials of the pigs were used later for inter-laboratory comparison tests and distribution of reference material, one of the main tasks of the EU Reference Laboratory. In order to obtain maximum information out of an animal experiment, several separate experiments, performed at different times, are evaluated together here. The set up of the experiments conducted by the EU Reference Laboratory is similar though not identical. Therefore some parameters do vary (e.g. number of pigs, breed and leukocyte count).

All pigs were kept under high containment conditions. Four groups of five eight week old German Landrace pigs were inoculated oronasally with 10^4 tissue culture infectious doses 50% ($TCID_{50}$) of the respective CSFV isolates CSF0695, CSF0650, CSF0634, and CSF0277. Four groups of four eight week old cross breed weaners (German Landrace x Pietrain) were inoculated oronasally with 10^4 $TCID_{50}$ of the respective CSFV isolates CSF0849, CSF0854, CSF0864, and CSF0870. Clinical examination and body temperature measurement were performed daily. Blood samples for haematological, serological and virological examinations were taken twice a week. Virus isolation on leukocytes and virus neutralisation tests to detect CSF antibodies were performed according to the EU Diagnostic Manual (Anonymus, 2002) and the Technical Annex accompanying it. The clinical signs were evaluated according to the clinical score developed by Mittelholzer et al. (2000) with slight modifications. Moribund animals were euthanized and a post mortem examination performed. The pathologically

120 important organs for the diagnosis of CSF were evaluated according to a pathological score
121 developed by Floegel-Niesmann et al. (2003). The clinical and pathological scores have a
122 scale from 0 - 3 points according to the severity of the lesion: score 0 = normal, score 3 =
123 severe CSF symptom. For the clinical score, these parameters were assessed daily, whereas
124 the pathological score could only be assessed on the day of euthanasia. The mean clinical
125 score was calculated from the highest score of each animal in each group. Selecting a defined
126 day for this calculation would be misleading, because animals which recover score lower
127 points with progressing time whereas others are already dead. The maximum score was 27
128 points for the clinical signs and 30 points for the pathological signs. Parameters evaluated for
129 the clinical signs were appetite, liveliness, body tension, shape, breathing, gait, eyes, skin, and
130 defaecation. In addition, three further parameters were included to evaluate the virulence of
131 the four CSFV: Case fatality at three weeks post infection, leukocyte counts between 0 and 14
132 days post infection (dpi) (Stegemann et al., 2000) and the homologue CSF antibody titre at 14
133 dpi. They were scored as follows: Case fatality 0% = 0 points, 1-40% = 1 point, 41-80% = 2
134 points and >80% = 3 points; leukocyte count: >10 G/l = 0 points, 8.6 – 9.9 G/l = 1 point, 6.5 –
135 8.5 G/l = 2 points and <6.5 G/l = 3 points; homologue CSF antibody titre: >5 ND50 = 0
136 points and <5 ND50 = 3 points. Points for these additional parameters were calculated into the
137 clinical score (CS), presenting now a modified CS (mCS). Classification is now made as
138 follows: > 18 points = highly virulent, > 6 points = moderately virulent, < 6 points low
139 virulent (see Table 1) (Bunzenthal, 2003).
140

141 **Results**

142 Regarding the incubation period, all six so far unknown CSFV had a shorter incubation period
143 (3-5 days) compared to CSF0634 and CSF0277 (6-8) days. The mean CS ranged between 10
144 and 17.6 points. The lowest CS was 10 for CSF0650 and the highest score 17.6 for CSF0634
145 (see Table 1). Regarding case fatality, in each group at least one pig died (see Table 1). Pigs

infected with CSF0634 and CSF0870, respectively, showed the highest case fatality of 100 %, whereas pigs infected with CSF0650 showed the lowest case fatality rate of 20 %. None of the pigs infected with CSF0634 and CSF0849 had a detectable homologue CSF antibody titre at 14 dpi. From pigs infected with CSF0277, CSF0854, CSF0864, and CSF0870, respectively, only one pig in each group started to produce CSF antibodies at 14 dpi against the homologue virus (neutralizing dose 50 % (ND_{50}) 15-30). CSF0650 and CSF0695 both induced CSF antibody titres between 10 and 80 ND_{50}. The leukocyte count decreased in all investigated CSFV infected pigs below 10 G/l (see Table 1). The leukocyte count for pigs infected with CSF0849, CSF0854, CSF0864, and CSF870 were not performed for technical reasons. CSF0634 and CSF0854 scored the highest pathological score (15.4 and 15.25 respectively), followed by CSF0849 (14.25). CSF0650 and CSF0277 scored rather similar (5.6 and 5 respectively) the lowest pathological scores. Pathological findings were most obvious in lymphnodes upon infection with all eight CSFV isolates (see Table 2). Scores for skin lesions, representing the classical haemorrhagic picture, ranged from 0.2 in CSF0650 up to 2.5 in CSF0849.

The mCS (see Table 1) clearly defines CSF0634, CSF0849 and CSF0854 as highly virulent, whereas CSF0864 and CSF870 are borderline between highly and moderately virulent. CSF0650, CSF0695 and CSF0277 were classified as moderately virulent. No CSFV was classified as low virulent.

Discussion

The purpose of this study was to assess six so far uncharacterized CSFV isolates from 1996 until 2007 for their virulence by using the recently developed pathological score and extending the existing clinical score by additional parameters. This included more parameters of clinical and laboratory diagnosis to characterise virulence more objectively and gain valuable information for clinical diagnosis of CSF and disease eradication.

Whether animal experiments performed by the EU Reference Laboratory giving a reference to all EU Member States should be done with inbreed pigs, available from SPF-holdings or cross-breed pigs which are actually found in the farms and will meet the field virus can be discussed in several ways. When deciding for cross-breed pigs, cross-breed fattening pigs from Southern Europe will not be identical to cross-breed fattening pigs from Northern Europe either. However, looking at CSF epidemics affecting several countries, the CSFV isolate never had difficulties infecting local pig populations (Elbers et al., 1999). Also wild boars get CSF infections in different parts of Europe and the virus enters the local domestic pig population (Fritzemeier et al., 2000). Depner et al., 1997 observed breed related differences during an experimental CSF infection with a different CSFV (CSF0123). It was a single experiment and reproduction turned out to be rather difficult (data unpublished) nor has any other scientific publication been made on the subject. Therefore the use of the pig breed should not be overestimated here.

The standard deviation between the pigs of each infected group for the clinical and pathological is rather high. This is not surprising, when some animals in each group do recover and others die showing clinical and pathological signs. In recovering pigs, clinical signs naturally are less pronounced and pathological signs are almost absent. The standard deviations for CSF0634 are the lowest because all animals had obvious clinical signs.

Regarding the introduction of the additional parameter case fatality, it did not always correlate with a high CS. Animals with the highest CS also had a high case fatality rate (CSF0634), however, CSF0870 with a case fatality rate of 100% had a clinical score of only 12.5. Furthermore CSF0277 had a lower CS than CSF0695 but a higher case fatality .

Consequently, high case fatality is not necessarily associated with a high CS. Therefore case fatality is an important parameter which needs to be included, when evaluating virulence.

CSF0277 had also been characterised in the marker vaccine trial performed by EU member states (Uttenthal et al., 2001). There, the case fatality differed in pigs from different countries.

198 However, a high case fatality is quite easy to observe in a pig farm and has to be regarded as
199 an individual sign for CSF independent from clinical signs. Consequently, it is essential to
200 include case fatality when evaluating virulence, which is achieved now in the new mCS.
201 Regarding antibody production against CSF, CSF695 and CSF650 both induced significant
202 CSF antibody titres at 14 dpi (see Table 1). This lowers the mCS and may lead to recovery of
203 infected animals with less obvious clinical signs. On the other hand, these CSF antibody titres
204 would be easy to detect in laboratory diagnosis using commercial ELISAs or neutralisation
205 tests Floegel-Niesmann et al. (2004) and helps identifying CSF infected animals which are
206 recovering.
207 Applying the new mCS, pigs infected with CSF0634 had the highest score in all parameters
208 and can now be characterised as highly virulent in the age class of weaner pigs. Previously
209 CSF0634 had been characterised as moderately virulent (Floegel-Niesmann et al., 2003).
210 The CSFV classified as highly virulent in the mCS had the highest total pathological score
211 (CSF0634, CSF0849, and CSF0854). This indicates that there is a correlation between the
212 pathological signs and the classification of virulence in the mCS: high virulence is associated
213 with increased pathological lesions. However, many pathological signs were non-specific
214 with lymphadenosis dominating the picture.
215 The mCS clearly shows that there are several clinical signs and laboratory diagnostic of blood
216 parameters which can be recognised during CSF infection and it should be possible to detect
217 some of them during clinical investigation in an infected farm of a new CSF outbreak. With
218 the introduction of the new mCS, speculations of mysteriously low virulent CSFV circulating
219 in pig populations could not be sustained. A subclinical course of CSF infection in weaner
220 pigs is therefore quite unlikely. It would be of scientific and epidemiological interest to
221 conduct the same experiments with pigs from different age groups eg. 50 kg fattening pigs,
222 100 kg fattening pigs and breeding sows. But animal welfare is given priority here and
223 therefore experiments with the most susceptible age group must be sufficient. However, the

individual signs in other age groups may be less typical. Especially in fattening pigs, where respriratory infections are rather common, it is likely that these dominate the clinical signs as misleading secondary infections.

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Table 1: Incubation period, Clinical Score (CS), additional parameters leukocyte count, case fatality, homologue CSF antibody titre at 14dpi , the new modified Clinical Score (mCS) calculated out of them and the classification of virulence for all investigated CSFV.

CSFV	Mean incubation period (days)	CS (Points) +/- (Standarddeviation)	Leukocyte count (G/l)	Case fatality at 22dpi (%)	highest antibody titre (ND_{50}) 14dpi	mCS (Points)	Virulence
CSF0650	4-5	10 (3,5)	8,5	20	80	13,6	moderate
CSF0695	3-4	13,4 (3,5)	7,5	40	40	17,7	moderate
CSF0634	6-8	17,6 (2,7)	5,8	100	<5	26,6	high
CSF0277	6	10,2 (4,6)	9,4	60	15	15,4	moderate
CSF0849	4-5	14 (3,7)	n.d.	50	<5	19	high
CSF0854	5	15,25 (4,6)	n.d.	25	20	19,5	high
CSF0864	4	13,75 (3,9)	n.d.	75	15	18	moderate/high
CSF0870	5	12,5 (6,1)	n.d.	100	30	17,75	moderate/high

n.d.: not done

Table 2: Mean pathological score (PS) of organs of five or four pigs for each CSFV at day of euthanasia of pigs. Standarddeviation between pigs infected with the respective CSFV is given.

Organ/CSFV	0650	0695	0634	0277	0849	0854	0864	0870
Skin	0,2	1,2	2,2	0,4	2,5	2,25	0,5	0,75
Serosae	0	1	2	0,4	0	0,75	0,5	0
Ln. inguinales	1,4	1,6	2,4	1,2	2,5	2	1,5	2
Ln. mandibulares	2	1,4	2,4	1	2,25	2	1,5	2
Ln. ileocaecalis	1,4	1,4	1,8	0,8	1,25	1,5	1	1,75
Tonsil	0	1	0	0,2	1,25	1,75	1,25	2
Spleen	0,2	0	0,4	0	0,25	1	0,75	0,25
Kidney	0,2	1	0,8	0	1,75	1,5	0,5	0,5
Ileum	0	0,8	2,2	0,4	0,75	1,25	0,5	0,5
Respiratory system	0,2	0	1,2	0,6	1,75	1,25	0,75	0,75
Total PS	5,6	9,4	15,4	5	14,25	15,25	8,25	10,5
Standarddeviation between pigs	3,7	10,26	1,14	2,9	3,69	6,87	3,86	2,65