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Short communication

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 to 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 was 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.

1. 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 to 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 (Mengeling 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), characterisation 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

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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.

2. Materials 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 (Vlasova 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.

3. 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 8-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 8-week-old cross-breed weaners (German Landrace $\times$ 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 (Anonymous, 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 important organs for the diagnosis of CSF were evaluated according to a pathological score developed by Floegel-Niesmann et al. (2003). The clinical and pathological scores have a scale from 0 to 3 points according to the severity of the lesion: score 0 = normal and score 3 = severe CSF symptom. For the clinical score, these parameters were assessed daily, whereas the pathological score could only be assessed on the day of euthanasia. The mean clinical score was calculated from the highest score of each animal in each group. Selecting a defined day for this calculation would be misleading, because animals which recover score lower points with progressing time whereas others are already dead. The maximum score was 27 points for the clinical signs and 30 points for the pathological signs. Parameters evaluated for the clinical signs were appetite, liveliness, body tension, shape, breathing, gait, eyes, skin, and defaecation. In addition, three further parameters were included to evaluate the virulence of the four CSFV: case fatality at 3 weeks post infection, leukocyte counts between 0 and 14 days post infection (dpi) (Stegeman et al., 2000) and the homologue CSF antibody titre at 14 dpi. They were scored as follows: case fatality 0% = 0 points, 1–40% = 1 point, 41–80% = 2 points and >80% = 3 points; leukocyte count: >10 G/l = 0 points, 8.6–9.9 G/l = 1 point, 6.5–8.5 G/l = 2 points and <6.5 G/l = 3 points; homologue CSF antibody titre: >5 ND50 = 0 points and <5 ND50 = 3 points. Points for these additional parameters were calculated into the clinical score (CS), presenting now a modified CS (mCS). Classification is now made as follows: