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# Characterisation of experimental infections of domestic pigs with genotype 2.1 and 3.3 isolates of classical swine fever virus

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ABSTRACT

The early identification of classical swine fever epizootics is hampered by difficulties in recognising early signs of infection, due to a lack of specific clinical signs. In addition many textbook descriptions of CSF are based on observations of disease caused by historic, mainly genotype 1, strains. Our objective was to improve our knowledge of the diverse range of signs that different CSFV strains can cause by characterising the experimental infection of domestic pigs with both a recent strain of CSFV and a divergent strain. Conventional pigs were inoculated with a genotype 2.1 isolate, that caused an outbreak in the UK in 2000, and a genotype 3.3 strain that is genetically divergent from European strains. This latter strain is also antigenically distinct as it is only poorly recognised by the CSFV-specific monoclonal antibody, WH303. Transmission was monitored by use of in-contact animals. Clinical, virological and haematological parameters were observed and an extended macro- and histopathological scoring system allowed detailed characterisation of pathological lesions. Infection with the genotype 2.1 isolate resulted in a similar outcome to other recent genotype 2 European strains, whereas the genotype 3.3 strain produced fewer and delayed clinical signs, notably with little fever. This strain would therefore be particularly difficult to detect in the early stages of infection and highlights the importance of encouraging early submission of samples for laboratory diagnosis. As representatives of recent and divergent CSFV isolates, these strains are good candidates to study the pathogenesis of current CSFV isolates and as challenge models for vaccine development.



## 1. Introduction

Although many areas of the world have eradicated classical swine fever (CSF), epizootics in disease-free regions occur regularly and cause considerable financial and sociological impact. The last outbreak in the UK, in 2000, resulted in the destruction of over 73,000 animals. Of these, 31,900 were culled pre-emptively as dangerous contacts, or due to their close proximity to confirmed cases, but were subsequently confirmed as not infected (Paton, 2002). Early detection is key to ensuring that control measures will minimise the spread and the impact of such devastating diseases (Murray and McCutcheon, 1999). Identification of CSFV as the etiological agent of some recent outbreaks has taken 2 months or more after the virus was introduced. For the UK2000 outbreak, it is estimated that the virus initially entered the index farm in early June, but confirmation of disease did not occur until August (Gibbens et al., 2000). A lack of pathognomonic

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signs linked to CSF is one of the factors contributing to delays in its recognition and it has been speculated that recent isolates, for which clinical diagnosis on infected farms was difficult, are of lower virulence (Terpstra and de Smit, 2000). Indeed, European isolates from the late 1990s produced less pronounced and delayed signs in experimental inoculations compared to the historical reference strain, Alfort (Floegel-Niesmann et al., 2003).

The UK2000 outbreak was caused by a genotype 2.1 CSFV, which is genetically distinct from previous European 2.1 isolates (Sandvik et al., 2000). The origin of this incursion could not be determined. However, it is thought that exposure of outdoor pigs to an illegally imported infected pork product was the most likely source (Gibbens et al., 2000). Other recent outbreaks, that have been caused by genotype 2.1 strains, such as in the Netherlands in 1997 (Greiser-Wilke et al., 2000) and in South Africa in 2005 (Sandvik et al., 2005), highlight that this genotype poses a continued threat to CSF-free countries.

Viruses belonging to genotype 3 have had a more restricted distribution, being predominately constrained to the Far East. Isolates of the sub-genotype 3.3 have, thus far, only been collected from Thailand. The nucleotide sequences of characterised 3.3 isolates are quite divergent, indicating that this sub-genotype may have evolved locally for some time (Parchariyanon et al., 2000). This genetic diversity is also reflected by antigenic heterogeneity: 21 isolates were classified into 7 different antigenic groups based on monoclonal antibody typing. In particular two isolates, CBR/93 and CBR/94/2, did not react with Mab WH303 that, to our knowledge, has previously reacted with all other CSFV strains tested (Parchariyanon et al., 2001). This antibody is widely used in differential diagnosis of pestiviruses as it binds an epitope in the E2 glycoprotein, TAVSPTTLR, which is conserved in CSFV but not in BVDV and BDV strains (Lin et al., 2000). The TAVSPTTLR epitope is a virulence determinant, as mutants with amino acid substitutions in this region produce mild clinical signs (Risatti et al., 2006), raising the possibility that viruses like CRB/93 or CBR/94/2 would be particularly difficult to detect.

This study aimed to characterise the clinical, virological, haematological and pathological features associated with infection of pigs with the UK2000/7.1 and CBR/93 isolates. The results add to the knowledge of the behaviour of modern and diverse strains of CSFV in vivo and establish a challenge model for future vaccination experiments. We have also used a modified pathological scoring system to aid the comparison of experimental infections with different strains of CSFV.

## 2. Materials and methods

### 2.1. Viruses

CSFV strain UK2000/7.1 (Sandvik et al., 2000) was isolated from a domestic pig during the UK2000 outbreak and passaged three times in cell culture. CBR/93 was isolated in Thailand in 1993 (Parchariyanon et al., 2000). Viruses were propagated in PK-15 cells as previously described (Drew, 2008). The E2 region of the CBR/93 strain was amplified by PCR using SuperScript III Reverse Transcriptase (Invitrogen) and KOD Hot start DNA polymerase (Novagen) and the nucleotide sequence determined by ABI sequencing.

### 2.2. Animals

Ten-week-old Large White/Landrace cross-breed pigs were obtained from a local commercial supplier. The pigs were clinically healthy and free of antibodies against pestiviruses. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. During the acclimatisation period, biothermal microchips (Destron Technologies Inc.) were implanted subcutaneously behind the left ear for temperature monitoring. Biochip and rectal temperatures taken at the start of the experiment indicated that biochip temperatures were approximately 1 °C lower than rectal temperatures. Throughout the duration of the study, temperatures and clinical signs were recorded twice daily, using a slightly modified version of a scheme described previously (Mittelholzer et al., 2000). The ten parameters, which were scored between 0 and 3, were the same as described except a parameter measuring the leftovers at feed was replaced by a clinical score (CS) for the biochip temperature (37.0–38.9 °C = CS 0, 39.0–39.9 °C = CS 1, 40.0–40.9 °C = CS of 2, 41.0 °C or above CS = 3).

### 2.3. Animal challenge

For each experiment, six pigs were inoculated by intranasal administration of 2 ml of CSFV tissue culture supernatant (1 ml in each nostril), using a MAD300 nasal drug aerosol delivery device (Wolfe Tory Medical Inc.). Back titration of inocula on PK-15 cells verified that $10^{5.6}$ $TCID_{50}$ of both strains was delivered. After 24 h, the inoculated pigs were re-introduced to three in-contact animals in another pen. For each experiment, two uninfected control animals were housed in a separate pen. EDTA blood samples were obtained prior to inoculation and then at 2–3-day intervals post infection. Nasal swabs were collected daily. Control animals were sampled at the same time points as inoculated animals. Any animal, with a clinical score above or approaching 15, was euthanized by administration of 20% pentobarbitol solution, for humane reasons.

### 2.4. Viral RNA quantification in nasal swabs and EDTA blood

Nasal swabs were soaked in 1 ml PBS, agitated and then centrifuged at 1500 rpm for 7 min. Blood samples were collected in EDTA vacutainers (BD Biosciences). Viral RNA was extracted from 140 μl nasal swab suspension or EDTA blood using QIAamp 96 DNA Swab BioRobot or QIAamp Viral RNA mini kits (Qiagen), respectively. Viral RNA was quantified using a qRT-PCR one-step Superscript III Platinum kit (Invitrogen). The reaction mix contained 3 μl nucleic acid extract, 12.5 μl 2× reaction mix, a final concentration of 5 mM $MgSO_4$, 0.2 μl RNAsin, 50 nM ROX and 1U Superscript III reverse transcriptase/Platinum Taq mix. Primers CSF100F and CSF192-R (Hoffmann et al.,