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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.

Keywords: Classical swine fever, genotypes 2.1 and 3.3, experimental infection

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 two months or more after the virus was introduced. For the UK 2000 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 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 1990's produced less pronounced and delayed signs in experimental inoculations compared to the historical reference strain, Alfort (Floegel-Niesmann et al., 2003).

The UK 2000 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.

Materials and methods

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.

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°C-38.9°C = CS 0, 39.0°C –39.9°C = CS 1, 40.0°C- 40.9°C = CS of 2, 41.0°C or above CS = 3)

Animal challenge

For each experiment, six pigs were inoculated by intranasal administration of 2ml of CSFV tissue culture supernatant (1ml 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 24h, 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 to 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.

Viral RNA quantification in nasal swabs and EDTA blood

Nasal swabs were soaked in 1ml PBS, agitated and then centrifuged at 1500rpm for 7min. Blood samples were collected in EDTA vacutainers (BD Biosciences). Viral RNA was extracted from 140µl 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 3µl nucleic acid extract, 12.5µl 2x reaction mix, a final concentration of 5mM $MgSO_4$, 0.2µl RNAsin, 50nM ROX and 1U Superscript III reverse transcriptase/Platinum Taq mix. Primers CSF100F and CSF192-R (Hoffmann et al., 2005) were used at a concentration of 600nM and the probe CSF-

Probe-1 , which was modified with BHQ1 quencher, was used at 200nM. The viral genome copy number was determined by comparison to a ten-fold serial dilution of a standard included in each PCR. This PCR standard consisted of RNA transcribed *in vitro* (Megashortscript kit, Ambion) from plasmid pCRXLV324-6 which contains the region of the 5'UTR of CSFV strain Alfort 187, flanked by primers V324 and V326 (Vilcek et al., 1994).

Haematology

To monitor thrombocytopenia, EDTA blood (500μl) was centrifuged at 300g for 1min and 5μl plasma were added to 2.5ml MACSQuant Running Buffer (Miltenyi Biotec). Platelets present in 50μl of this suspension were identified based on forward and side scatter characteristics using a MACSQuant flow cytometer (Miltenyi Biotec). Leukocytes present within 50μl of EDTA blood were detected by staining with 10μl of a porcine pan-leukocyte anti-CD45-FITC antibody (AbD Serotec). Samples were then mixed with 940μl FACS lysing solution (BD Biosciences), to ensure lysis of erythrocytes and fixation of cells, prior to assessment of total leukocyte counts in 50μl of the cell suspension using a MACSQuant flow cytometer.

Pathology

Post mortem examinations followed standard operational procedures and any observed lesions were recorded. A macropathology scoring system was established (Table I) using a total of 21 parameters. Each parameter was evaluated from 0 (no lesion) to 3 (severe lesion). Samples were fixed in buffered formalin and embedded in paraffin wax. Sections (4μm) were stained

with H&E for histopathological examination. A histopathological scoring system was also developed using 33 parameters (Table II) and the severity of lesions was also recorded from 0 (no lesion) to 3 (severe). Data from both scoring systems were analyzed by Kruskal-Wallis non-parametric mean comparison test and differences were considered significant for $P<0.05$.

Results

Characterisation of CSFV CBR/93

To confirm that the CBR/93 isolate is variant at the WH303 epitope, PK-15 cells infected with CSFV strain UK2000/7.1 or CBR/93 were stained and analysed by flow cytometry. Whereas UK2000/7.1 was easily detected with Mab WH303 staining of strain CBR/93 was impaired (data not shown). Sequence analysis of the E2 region revealed a nucleotide substitution that encodes a serine instead of a proline in the relevant amino acid sequence (TAVS**P**/**S**TTLR) (Accession number FJ790771).

Clinical features of UK2000/7.1 and CRB/93 infection

Upon inoculation with the UK2000/7.1 strain, two of the animals displayed clinical signs from 7-8 days post inoculation (dpi). The disease then progressed and these two animals were euthanized 13 dpi when their clinical scores were above 15 (Fig 1A). Onset of clinical signs was delayed slightly in the remaining four inoculated animals with scores above 4 occurring at 14 dpi, after which they steadily increased until 17 -18 dpi when the animals were euthanized. The three in-contact animals had initial clinical signs at 16 dpi; the

clinical scores were of a similar, but delayed, profile relative to the inoculated animals. The biochip temperatures correlated well with the clinical scores (Fig 1C). The most prominent clinical signs were a reddening of the conjunctiva and soft faeces or diarrhoea. These early signs were followed by loss of appetite, lethargy, stiffness of gait and preputial oedema. Notably, no reddening, haemorrhaghes or petechiae of the skin were observed in animals infected with UK2000/7.1 during the duration of the experiment, apart from a diffuse purplish discolouration of the abdominal skin of one of the pigs euthanized on 13 dpi.

In pigs inoculated with the CBR/93 strain, clinical signs were delayed and less severe (Fig 1B). Only one animal had signs resulting in a score above 5 before 15 dpi. The disease then progressed rapidly with two of the animals dying unexpectedly overnight on 18 and 19 dpi and three of the remaining four inoculated animals were euthanized at that point. One pig developed few signs and appeared to be recovering from infection towards the end of the experiment on day 20. Two of the in-contact animals developed mild clinical signs from dpi 18. In addition to the less severe and delayed signs, it was notable that CBR/93 inoculated animals had only a mild, rather insignificant increase in temperature from 14 dpi (Fig 1D). Diarrhoea was again a predominant sign as was lack of attentiveness. With this strain however, we observed a reddening of the skin, particularly of the ears, but very little conjunctivitis.

Viraemia and nasal shedding

In both experiments viral RNA was detected in EDTA blood of all but one of the inoculated animals by 4 dpi, with the viral load increasing to a peak at 11 dpi (Figure 2A, B). One of the UK2000/7.1 inoculated animals, which developed early clinical signs, was viraemic by 2 dpi and had a higher viral RNA load than the other UK2000/7.1 inoculated animals. On average the viral RNA copy number in blood was 1 $\log_{10}$ lower in CBR/93 than UK2000/7.1 inoculated animals between dpi 6 and 11. The pig that developed few clinical signs, following inoculation with CBR/93, became viraemic at the same time as the other CBR/93 inoculated animals, but the viral RNA load did not reach the same level and started to decrease from 11 dpi. In the case of both strains, the in-contact animals became viraemic at 14 dpi, 3-4 days before clinical signs developed (Fig 2A, B).

Viral RNA was detected in nasal swabs from all inoculated animals between 5 and 8 dpi; 2-3 days after the animals became viraemic, but before the onset of clinical signs (Fig2A, B). Viral RNA in nasal swabs then increased before reaching a plateau at 14 dpi. The viral load in nasal swabs of CBR/93 inoculated-animals was lower than with UK2000/7.1. Interestingly, low levels of viral RNA were present in the nasal swabs of the in-contact animals shortly after the inoculated animals started shedding, but before these in-contacts became viraemic. This low level persisted, then increased 2 days after the animals became viraemic.

Haematology

Animals, inoculated with either virus, developed thrombocytopenia (Fig 2C, D). Platelet counts decreased below the levels in uninfected control animals

from 6 dpi for UK2000/7.1 inoculated animals, whereas a difference between inoculated and control animals was only observed after 11 dpi for CBR/93 inoculated animals, when the reduction in platelets was then more severe. Animals also developed leukopenia (Fig 2E,F) with leukocyte counts decreasing below levels in control animals from 4 dpi.

Pathology

Animals inoculated with UK2000/7.1 and CBR/93 isolates had a generalized bilateral lymphadenopathy. The mandibular, lateral and medial retropharyngeal lymph nodes were most affected (Supplemental Figures S1, S2), followed by prescapular and ventral cervical superficial and, to a lesser extent, precrural, ileocaecal and inguinal superficial lymph nodes. Other frequently observed lesions were hydrothorax, hydropericardium, ascitis, secondary bacterial bronchopneumonia and presence of necrotic foci and diphteroid plaques in the ileocaecal valve and colon. Splenic infarcts and petechia in different organs were only observed on very few occasions. The in-contact animals had similar, but less severe lesions, than inoculated animals. Only minimal background lesions were found in control animals.

The most common histopathological finding observed in animals inoculated with UK2000/7.1 and CBR/93 strains was a mild to severe lymphoid depletion in the lymph nodes accompanied by hyperemia and haemorrhages. Different degrees of lymphoid depletion were also observed in thymus and spleen. Inflammatory lymphohistiocytic infiltrates were frequently observed as perivascular cuffing in the liver, kidney and encephalon. In the ileocaceal valve and colon, the findings consisted of ulcerative necrotizing colitis with

fibrin deposition. The most commonly observed lung lesions were acute bacterial bronchopneumonia and alveolar and interstitial oedema. The uninfected control animals did not show significant changes.

When the results of each group were compared using the macro and histopathological scoring systems no statistical differences were found between UK2000/7.1 and CBR/93 inoculated or UK2000/7.1 and CBR/93 in-contact animals. Significant differences were observed between the 3 groups (inoculated, in-contact and control) within the same experiment ($P<0.05$) (Figure 3).

Discussion

The clinical signs and outcomes of CSFV infections vary considerably depending on inherent properties of the virus strain as well as host and environmental factors. It is acknowledged that classifying the spectrum of different strains of CSFV into categories of high, moderate or low virulence is difficult. A system for assigning virulence based on the clinical score (CS >15 and temp >41°C as a highly virulent strain, and CS of 5 to 15 as moderately virulent) has been suggested (Mittelholzer et al., 2000) and is used by many laboratories. The system is useful to provide comparability between experiments. It is, however, difficult to assign the two strains used in this study according to the parameters of this system. Although two of the animals inoculated with UK2000/7.1 reached scores >15 by 13 dpi, the remaining inoculated or in-contact animals were euthanized before reaching a clinical score of 15 for welfare reasons. However, although these remaining animals may have progressed to reach scores above 15, the course of the disease

was certainly delayed compared to the outcomes described upon infection with strains generally considered as highly virulent such as Brescia, Eystrup (Mittelholzer et al., 2000) and ISS/60 (Belak et al., 2008). The disease seen with the UK2000/7.1 isolate more closely resembled the course of infection observed with recent 2.1 and 2.3 isolates (Floegel-Niesmann et al., 2003). Classification of UK2000/7.1 as a moderately virulent strain is therefore appropriate and is consistent with anecdotal observations in the field during the 2000 outbreak that the strain appeared less virulent than historic isolates. Assessment of the clinical signs caused by CBR/93 in the first 2 weeks would result in the strain being described as having low virulence. However the rapid progression of disease at 17 dpi, resulting in unexpected death of two animals, and a similar degree of pathology at post mortem compared to UK2000/7.1 categorizes this virus in the moderately virulent group. Importantly, these observations highlight the difficulty in the clinical diagnosis of CSF. In particular clinical signs such as high fever and skin lesions classically associated with the disease may not be present in these early stages.

Although clinical signs were not obvious, high levels of viral RNA were easily detected in both blood and nasal swabs during these early stages of infection. Advances in real time RT- PCR and automation of RNA extraction in the last few years open up more possibilities for large-scale and rapid laboratory testing for viral nucleic acid. It would therefore be beneficial if samples were submitted early for laboratory tests, if only to exclude the possibility of CSFV infection.

The use of biochips for monitoring temperatures provided practical advantages in terms of ease of monitoring which reduced disturbance of the animals and allowed more frequent measurements. This highlighted the importance of taking temperatures at consistent times as the animals had a marked circadian rhythm. It is possible that hyperthermia would be more apparent with the CBR/93 strain if rectal temperatures were monitored. However separate assessment of biochip verses rectal temperatures over a longer period indicate that, although the actual values are different, changes in temperature monitored by the two methods correlate well (unpublished data).

Despite the differences observed in the clinical signs, infection with both isolates resulted in similar pathological scores. The in-contact animals exposed to both strains were less affected pathologically. This is likely due to a delay in the initial infection although the different dose of infection is also likely to have some effect on the course of disease. The most prominent lesion observed was a generalized lymphadenopathy. Other lesions, such as pinpoint haemorrhages in the kidney or splenic infarcts in the spleen, which are sometimes considered to be pathognomonic of CSF, were observed on only very few occasions. Similar lesions to those observed can be found in animals with PDNS and virulent PRRS, again highlighting the need for early considerations of laboratory testing.

Comparing the virological and haematological parameters many were slightly, although not dramatically, lower and delayed with CBR/93 in comparison with UK2000/7.1. Leukopenia and thrombocytopenia were delayed and the level of viral RNA was lower in the blood during the second week post infection.

These observations may be linked with the fewer and delayed clinical signs observed with the CBR/93 strain. Preliminary data suggests that there is also a difference in the infection of peripheral blood cells between the two viruses (unpublished data) which may have implications for the clinical outcomes observed. It is also interesting that the mutant virus T3v (Risatti et al., 2006), in which a targeted change replaces the proline in the WH303 epitope, also resulted in delayed clinical signs with reduced viraemia and shedding compared to its' parental strain. This suggests that the delayed and less severe clinical signs observed with CBR/93 may be a result of the changes in the WH303 epitope rather than this being a feature of genotype 3.3 strains in general. However, further studies would be needed with other genotype 3.3 strains to confirm this.

The WH303 epitope on the E2 protein is an immunodominant site, the presence of which is widely utilised as part of the differential diagnosis of CSFV (Zhang et al., 2006). The natural occurrence of CSFV that do not react with this antibody should therefore be heeded during differential diagnosis and highlights that results from procedures reliant on a single CSFV-specific monoclonal antibody must be interpreted with caution. It also raises questions concerning the efficacy of conventional vaccines against such strains.

One of the aims of this study was to establish relevant challenge models for future vaccination and pathogenicity studies. The similarities of the clinical outcome with UK2000/7.1 to other recent genotype 2 isolates and the antigenic and genetic diversity represented by the CBR/93 isolate highlight that these two strains represent relevant challenge models of current CSFV isolates. Both viruses were consistently transmitted to in-contact animals,

which will allow prevention of shedding by vaccination to be assessed. The extended macro- and histopathological scoring systems described here will allow systematic comparison and assessment of pathological findings related to CSF infection.

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Conflicts of interest

None

Table I.
Macropathology parameters for post mortem evaluation of pigs infected with Classical swine fever virus. Scores from 0 (no lesion) to 3 (severe lesions).

Parameter	Score		
	+ (1)	++ (2)	+++ (3)
Body condition	Fair	Poor	Emaciated/cachectic
Skin and subcutis	Erythema	Hemorrhages in several places	Multiple generalized hemorrhages
Tonsils	Hyperemia	Focal necrosis	Multifocal/generalized necrosis
Fluid in body cavities	Ascitis or hydrothorax or hydropericardium	Fluid in 2 cavities	Acsitis, hydrothorax and hydropericardium
Spleen	Enlargement/splenomegaly	Individual infarction	Multiple infarcts
Kidney	Individual petechiae	Several petechia	Multiple petechia and discoloration
Ileum and ICV	Single serosal hemorrhages or hyperemia	Several hemorrhages and/or necrosis	Multiple necrotic foci
Caecum and colon	Hyperemia	Single necrotic foci	Multiple necrotic foci
Brain and meninges	Mild hyperemia	Moderate hyperemia	Fibrinopurulent meningitis
Respiratory system	Mild bronchitis/rhinitis	Single lobe bronchopneumonia or pleuritis	Multiple lobes bronchopneumonia and pleuritis
Lymph nodes*	Mild enlargement and/or hyperemia/petechiae	Moderate enlargement and hyperemia/petechiae	Severe enlargement and hyperemia/petechia
Unrinary bladder	Single petechiae	Multiple hemorrhages	Confluent ecchimotic haemorrhages
Thymus	Mild atrophy	Moderate atrophy	Severe atrophy
Conjunctiva	Mild serous conjunctivitis	Moderate seropurulent conjunctivitis	Severe purulent conjunctivitis

*Lymph nodes: precrural, inguinal superficial, mandibular, lateral retropharyngeal, medial retropharyngeal, prescapular, ventral cervical superficial and ileocaecal.
ICV = ileocaecal valve

Table II.
Histopathology parameters for evaluation of pigs infected with Classical swine fever virus. Scores applied for each lesion are: 0, no lesion; 1, mild; 2, moderate; 3, severe

Tissue	Parameters scored
Tonsils	Lymphoid depletion Hyperemia/hemorrhages
Spleen	Lymphoid depletion Hemorrhages
Thymus	Lymphoid depletion
Lymph nodes*	Lymphoid depletion Hemorrhages/hyperemia
Liver	Periportal lymphplasmacytic infiltrates
Lung	Alveolar/septal edema Acute Bronchopneumonia
Kidney	Hyperemia/hemorrhages Inflammatory infiltrates
Encephalon	Perivascular cuffing Meningitis
Ileocaecal valve	Lymphoid depletion Ulceration/Necrosis
Colon	Lymphoid depletion Ulceration/Necrosis
Bone marrow	Hypocellularity

*Lymph nodes: precrural, inguinal superficial, mandibular, lateral retropharyngeal, medial retropharyngeal, prescapular, ventral cervical superficial and ileocaecal.

Figure legends

Fig 1

Clinical scores and temperatures following experimental infection with UK2000/7.1 and CBR/93 viruses.

Mean clinical scores of inoculated, in-contact, and controls of animals following infection with UK2000/7.1 (A) and CBR/93 (B). For UK2000/7.1 two groups of inoculated animals, which displayed different kinetics in the onset of clinical signs, are plotted separately. Note that due to the low clinical scores observed upon CBR/93 infection results are plotted on a different scale. Mean biochip temperatures of inoculated, in-contacts or control animals following infection with UK2000/7.1 (C) or CBR/93 (D), error bars represent SEM.

Fig 2

Virological and haematological parameters following experimental infection with UK2000/7.1 and CBR/93 viruses.

Viraemia and nasal shedding of animals infected with UK2000/7.1 (A) and CBR/93 (B). The mean copy number of viral genome per μl of EDTA blood or nasal swab suspension in inoculated and in-contact animals as determined by qRT-PCR. Data for the CRB/93 inoculated animal that showed few clinical signs are excluded. Mean platelet counts, determined by flow cytometry, in animals inoculated with the UK2000/7.1 (C) and CBR/93 (D) viruses and compared to two uninfected control animals. Leukocyte counts were similarly monitored in the inoculated and control animals in the UK2000/7.1 (E) and CBR/93 (F) experiments by determining the mean total leucocyte count per μl. Results are the mean data for each group of pigs. Error bars represent SEM.

Fig 3

Macropathology (A, B) and histopathology (C, D) scores following experimental infection with UK2000/7.1 (A, C) and CBR/93 (B, D). Boxes represents percentile 25 to 75; whiskers represents maximum and minimum values; lines represent medians.

Supplemental Figure legends

Figure S1

Examples of macropathology and histopathology scoring, following experimental infection with CSFV UK2000/7.1 (A, B, C) and CBR/93 (D, E, F). A Haemorrhagic lymphadenitis in lateral retropharyngeal lymph node (score 3); B Button ulcers in the ileacaecal valve and colon (score 3); C Perivascular cuffings in the brainstem (score 3). Original magnification 100x; D Petechial and ecchymotic haemorrhages in the subcutaneous tissue (score 3); E Areas of bronchopneumonia in the apical lung lobes (score 3); F Severe thymic atrophy with loss of lymphocytes and no separation between cortex and medulla (score 3). Original magnification: 100x.

Figure S2

Examples of scoring of macropathological and histopathological changes following experimental infection with CBR/93 in lateral retropharyngeal lymph nodes from inoculated (A, D), in-contact (B, E) and uninfected controls (C, F) . A. Severe enlargement and hyperemia (score 3); B. Moderate enlargement and hyperemia (score 2). C. Normal (score 0). D. Severe haemorrhages and hyperemia (score 3) and moderate lymphoid depletion (score 2); E. Mild haemorrhages and hyperemia (score 1) and moderate lymphoid depletion (score 2); F. No hyperemia/haemorrhages (score 0) and no lymphoid depletion (score 0).

Fig 1

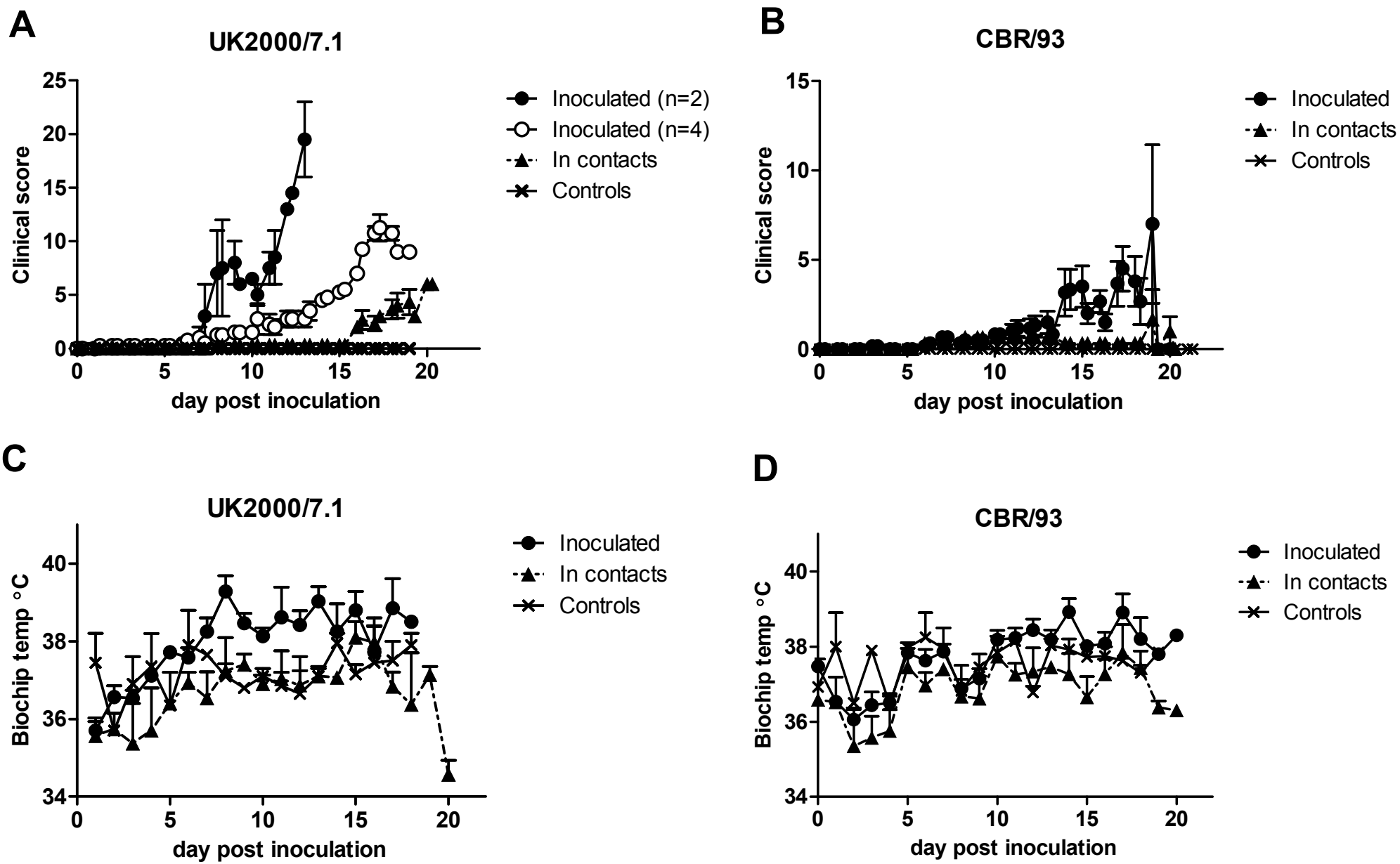
A
UK2000/7.1
Clinical score
day post inoculation
Inoculated (n=2)
Inoculated (n=4)
In contacts
Controls
B
CBR/93
Clinical score
day post inoculation
Inoculated
In contacts
Controls
C
UK2000/7.1
Biochip temp °C
day post inoculation
Inoculated
In contacts
Controls
D
CBR/93
Biochip temp °C
day post inoculation
Inoculated
In contacts
Controls

Fig 2

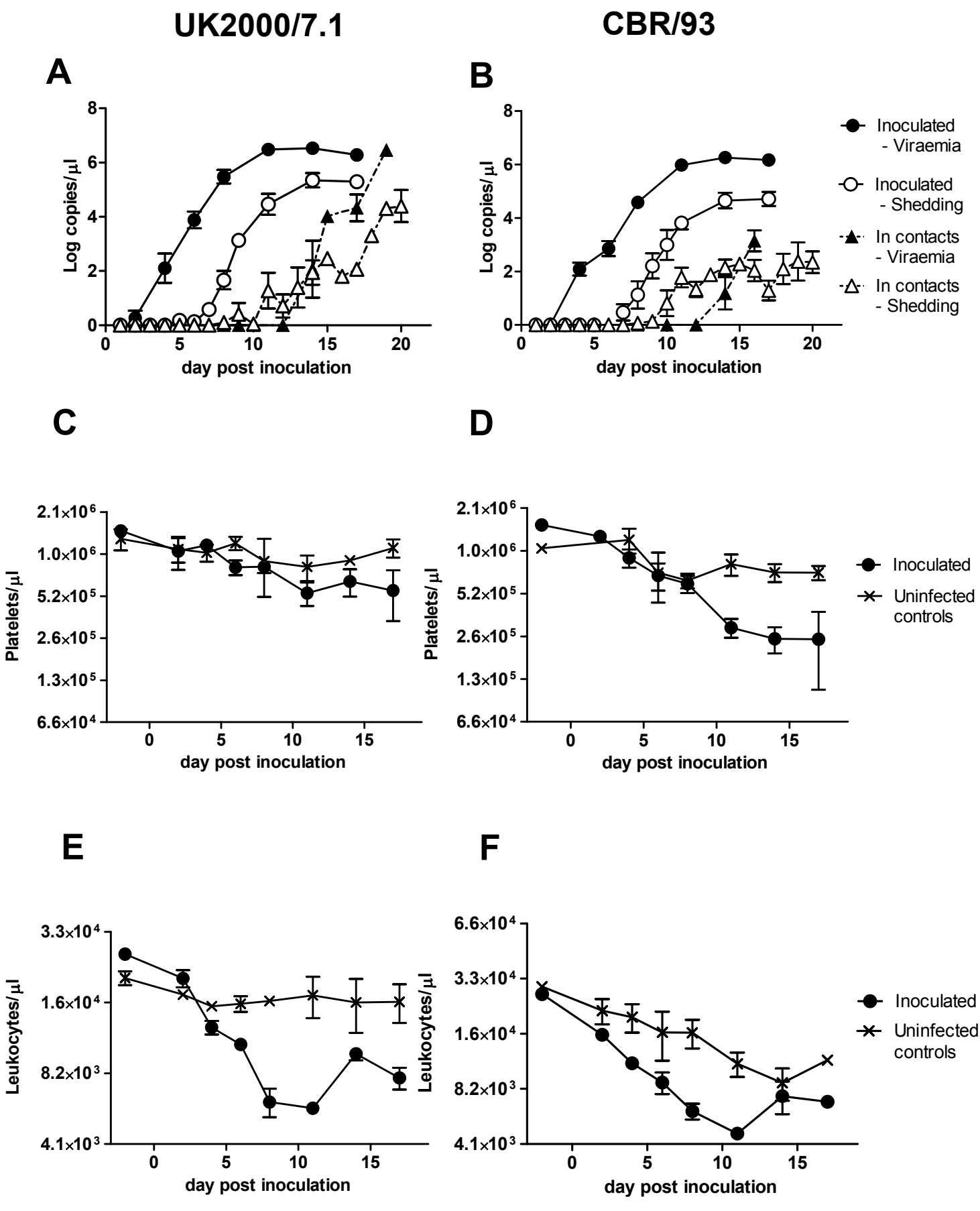
UK2000/7.1
CBR/93
A
B
Log copies/µl
day post inoculation
Inoculated - Viraemia
Inoculated - Shedding
In contacts - Viraemia
In contacts - Shedding
C
D
Platelets/µl
2.1×10⁶
1.0×10⁶
5.2×10⁵
2.6×10⁵
1.3×10⁵
6.6×10⁴
Inoculated
Uninfected controls
E
F
Leukocytes/µl
3.3×10⁴
1.6×10⁴
8.2×10³
4.1×10³

Fig 3