Antigenic characterization of classical swine fever virus YC11WB isolates from wild boar

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Classical swine fever (CSF), a highly contagious disease that affects domestic pigs and wild boar, has serious economic implications. The present study examined the virulence and transmission of CSF virus strain YC11WB (isolated from a wild boar in 2011) in breeding wild boar. Virulence of strain YC11WB in domestic pigs was also examined. Based on the severe clinical signs and high mortality observed among breeding wild boar, the pathogenicity of strain YC11WB resembled that of typical acute CSF. Surprisingly, in contrast to strain SW03 (isolated from breeding pigs in 2003), strain YC11WB showed both acute and strong virulence in breeding pigs. None of three specific monoclonal antibodies (7F2, 7F83, and 6F65) raised against the B/C domain of the SW03 E2 protein bound to the B/C domain of strain YC11WB due to amino acid mutations (720K→R and 723N→S) in the YC11WB E2 protein. Although strains YC11WB and SW03 belong to subgroup 2.1b, they had different mortality rates in breeding pigs. Thus, if breeding pigs have not developed protective immunity against CSF virus, they may be susceptible to strain YC11WB transmitted by wild boar, resulting in severe economic losses for the pig industry.

Keywords: B/C domain, Classical swine fever virus, Sus scrofa, virulence

Introduction

Classical swine fever virus (CSFV) belongs to the genus Pestivirus within the family Flaviviridae [10]. It is an enveloped virus with a single-stranded, positive-sense RNA genome that encodes a 3,898 amino acid (aa) polyprotein that undergoes co-translational and post-translational processing by cellular and viral proteases to yield four structural (C, Em, E1, and E2) and eight nonstructural (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [17]. Glycoprotein E2 is the most immunodominant protein and induces the production of neutralizing antibodies in infected pigs [21]. Four antigenic domains (A–D) have been identified in the N-terminal half of E2, which comprises two independent antigenic units: B/C and D/A [8,14,32]. Domain B/C, which comprises the 90 N-terminal residues, is responsible for the antigenic specificity of various CSFVs [20].

Acute classical swine fever (CSF) occurs mainly in young animals and is characterized by high fever, lack of appetite, conjunctivitis, and constipation; these symptoms are often followed by diarrhea, neurological signs, and hemorrhage of the skin and other organs, possibly accompanied by severe thrombocytopenia and leukopenia [15,25,27,30]. Host-virus interactions involve many factors (host age, genetic background, immune status, herd sanitary status, and strain virulence), and such interactions can lead to different clinical outcomes [11,19,26].

Since 2002, CSF in Korea has undergone an antigenic shift from genotype 3 to genotype 2 [29]. Recently, two virus isolates (strains YC11WB and PC11WB) were obtained from Korean wild boar captured during a campaign conducted for CSFV surveillance; these strains were identified as genotype 2.1b [29]. Comparative analysis of the nucleotide sequences of YC11WB Erns, E1, E2, and NS5B, along with those of a reference strain (SW03), revealed high sequence homology: 94.7% for the Erns genes, 94.4% for the E1 genes, 94.5% for the E2 genes, and 95.2% for the NS5B genes [3].

The aim of the present study was to compare the clinical signs, pathological lesions, viremia, virus shedding, and mortality among breeding pigs infected by strain YC11WB.
B/C and D/A domain-binding mAbs cultured under the following conditions: 1 mM isopropyl-β-D-1-thiogalactopyranoside (ITPG), 30°C, for 18 h (B/C protein); 0.5 mM IPTG, 37°C, for 5 h (D/A protein); or 1 mM IPTG, 25°C, for 18 h (ABCD protein).

Cloning and expression of the E2 protein domains

Total RNA was purified from strain LOM by using an RNeasy kit (Qiagen, USA) and cDNA was amplified using the SuperScript III First-Strand Synthesis System (Invitrogen, USA). DNA fragments of the antigenic domains (B/C, D/A, and ABCD) were amplified and cloned into vectors pET30a or pRSET to construct pET30a-LOM-E2/BC, pRSET-LOM-E2/DA, and pET30a-LOM-E2ABCD. These plasmids were then used to express the E2 protein domains recognized by the mAbs.

Materials and Methods

Viruses

Strain YC11WB (GenBank accession KC149990) belongs to genotype 2.1b and was isolated from a Korean wild boar in 2011 [19]. Strain SW03 (belonging to epidemic genotype 2.1b), a representative virulent strain from Korea, was isolated from a breeding pig farm in 2003. Strain LOM (genotype 1) was derived from a low virulence strain and used to develop an attenuated live vaccine virus, which has been used in Korea for 40 years.

Cloning and expression of the E2 protein domains

Fifteen mAbs specific for the E2 protein of the LOM strain were produced in-house or donated by Median Diagnostics (Korea), which manufactures veterinary diagnostic kits. The remaining mAb, WH303, which binds specifically to the D/A domain, was obtained from APHA (UK). The three virus strains (LOM, YC11WB, and SW03) were inoculated into porcine kidney-15 cells, which were then examined 3 days later using mAbs specific for the B/C or D/A domains to detect antigenic differences between strains. The B/C, D/A, and ABCD domains expressed by the plasmids pET30a-LOM-E2/BC, pRSET-LOM-E2/DA, and pET30a-LOM-E2ABCD plasmids, respectively, were then examined by performing western blotting to identify the protein regions recognized by the mAbs.

Predicting the three-dimensional structure of the E2 protein

The sequences of the CSFV strains were obtained from GenBank via the National Center for Biotechnology Information (USA) website. The three-dimensional (3D) structures of the CSFV E2 proteins were predicted by using the RaptorX program [23]. The putative epitope within the E2 protein was predicted by using the Emini Surface Accessibility Prediction program [9] and BepiPred Linear Epitope Prediction [16].

Animals experiment using strains YC11WB and SW03

The test animals were separated into six experimental groups. Group 1, comprising two breeding wild boar, was used to verify the virulence of strain YC11WB. Group 2 comprised two breeding wild boar housed with the infected breeding wild boar from Group 1. These animals were used to examine contact-mediated transmission. Group 3 comprised two Landrace pigs, which were used to check the virulence of strain YC11WB. Group 4 comprised two Landrace pigs infected with strain SW03 to compare pathogenicity, used as positive control. Groups 5 and 6 comprised two breeding wild boar and two Landrace pigs, respectively (negative controls). All Landrace pigs and breeding wild boar were approximately 40 days old and 70 days old, respectively, and all were negative for anti-CSFV antibodies. All animals weighed about 14 kg (n.b.), the 30 day age difference between the Landrace pigs and the breeding wild boar is due to the fact that breeding wild boar grow slowly. Both virus strains were used at the same dosage in the challenge experiments (10^{6.0} TCID_{50}/mL). Pigs were monitored daily until 21 days post-infection (DPI), mainly to record body temperature, and mortality was monitored. Pig samples used for virological, serological, hematological, and pathological analyses were collected at different intervals (2 or 3 days).

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay of samples

Samples (blood, nasal swabs, saliva, and feces) were collected from the experimental animals at 3, 5, 7, 9, 11, 14, 17, and 21 DPI. Necropsies were performed and tissue samples were obtained from 13 organs: tonsil, lung, heart, spleen, liver, mesenteric lymph node, kidney, ileum, cecum, inguinal lymph node, cerebrum, cerebellum, and spinal cord. Total RNA was extracted from all samples by using a micro-column-based QIAamp Viral RNA Mini Kit (Qiagen). The CSFV real-time RT-PCR assay was based on primers and probe developed at the Plum Island Disease Center. The nucleotide sequences of the primers and probe targeted the highly conserved 5′ untranslated region of the CSFV genome. The primer and probe sequences were as follows: TGCCCAAGACACACCTTAACC (forward primer), GGCTCTGCAAGCCCTAT (reverse primer), and FAM-TGATGGGAAGTACGACCTG-MGBNFQ (probe) [5]. The thermocycling conditions for amplification were as follows: one cycle of RT at 50°C for 30 min and one cycle of heat activation at 95°C for 15 min, followed by 45 cycles of PCR amplification (heat denaturation at 94°C for 15 sec and extension at 56°C for 60 sec). Amplification was performed in
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Table 1. Reactivity of a panel of monoclonal antibodies with vaccine and virulent strains of classical swine fever virus (CSFV)

| Domain | Anti-CSFV antibody | Western blot result | Indirect fluorescence assay (IFA) result |
|--------|--------------------|---------------------|----------------------------------------|
|        |                    | pET30a-LOM-E2/BC     | pRSET-LOM-E2/DA | pET30a-LOM-E2/ABCD | LOM (genotype 1) | SW03 (genotype 2.1b) | YC11WB (genotype 2.1b) |
| B/C    |                    |                     |                     |                    |                   |                     |                          |
| 6H10   | +                  | −                   | −                   | −                   | +                  | + + + +               | + + + +                   |
| 6H1    | +                  | −                   | −                   | −                   | +                  | + + + +               | + + + +                   |
| 7F2*   | +                  | −                   | +                   | +                   | +                  | + + + +               | −                        |
| 7F34*  | +                  | −                   | +                   | +                   | +                  | + + + +               | −                        |
| 6F65*  | +                  | −                   | +                   | +                   | +                  | + + + +               | −                        |
| 1A39   | + + + +            | −                   | +                   | +                   | + + + +            | −                    | −                        |
| 10A56  | + + + +            | −                   | +                   | +                   | + + + +            | −                    | −                        |
| D/A    |                    |                     |                     |                    |                   |                     |                          |
| 4C29   | −                  | + +                | + +                | + +                | + + + +            | +                    | +                        |
| 6A92   | −                  | + + + +            | + + + +            | + + + +            | + + + +            | + + + +               | +                        |
| 2-21-9 | −                  | + +                | + +                | + +                | + + + +            | + + + +               | +                        |
| 45-43  | −                  | + +                | + +                | + +                | + + + +            | + + + +               | +                        |
| 2-187  | −                  | + + + +            | + + + +            | + + + +            | + + + +            | + + + +               | +                        |
| 4F69   | −                  | + + + +            | + + + +            | + + + +            | + + + +            | + + + +               | +                        |
| 4F41   | −                  | + + + +            | + + + +            | + + + +            | + + + +            | + + + +               | +                        |
| WH303  | −                  | + + + +            | + + + +            | + + + +            | + + + +            | + + + +               | +                        |

*Antibodies that reacted only with strain SW03 and LOM. +, week; + +, moderate; + + +, strong; −, negative reaction.
in Group 2) wild boar at 3 and 5 DPI, respectively (Table 2). Analysis of blood, nasal, fecal, and salivary fluid samples from animals in Group 3 revealed that all pigs were positive at 5 DPI (Table 2). At 5 DPI, blood samples from pigs in Group 4 showed evidence of viremia and fecal samples showed evidence of viral shedding.

### Table 2. Detection of viral RNA and virus shedding in Classical swine fever virus-infected pigs by reverse transcriptase-polymerase chain reaction

| Group | 1 | 2 | 3 | 4 | 5 | 6 |
|-------|---|---|---|---|---|---|
| Cs    | YC11WB | YC11WB | YC11WB | SW03 | Non-treatment | Non-treatment |
| Ps    | Wild boar | Wild boar | Landrace pig | Landrace pig | Wild boar | Landrace pig |
| Pn    | W26 | W28 | NW32 | NW35 | R110 | R112 |
| 0*    | a | a | a | ac | c | - |
| 3     | abcd | abcd | a | bd | abcd | abcd |
| 5     | abcd | abcd | abd | bcd | abcd | abcd |
| 7     | abcd | abcd | abc | abd | abcd | abcd |
| 9     | abcd | abcd | abc | abd | abcd | abcd |
| 11    | abcd | abcd | acd | abc | abcd | abcd |
| 14    | abcd | abcd | abc | abd | abcd | abcd |
| 17    | abcd | abcd | abc | abd | abcd | abcd |
| 21    | abcd | abcd | abc | abd | abcd | abcd |
| D     | 17 | 18 | 18 | 21 | 10 | 13 |

*0 days post-infection. Cs, challenge virus strain; YC, YC11WB; SW, SW03; Ps, pig species; Pn, pig number; -, negative; a, blood; b, nasal swab; c, fecal; d, salivary fluid; D, death date (the figure in parentheses indicates the date of death [expressed as days post-infection]).

### CSFV RNA copy number in tissue samples

Results of RT-PCR of 13 tissue samples taken from wild boar W26 and W28 revealed between 1.49 and 6.87 log_{10} copies of CSFV RNA. Tissue samples from wild boar NW32 and NW35 in Group 2 (exposed to contact-mediated transmission from wild boar in Group 1) harbored between 1.06 and 6.38 log_{10} copies (Table 3). When Landrace pigs were infected with strain YC11WB, animals R110 and R112 in Group 3 harbored a high number of CSFV RNA copies in tonsil and lung tissues; however, SW03 RNA was rarely detected in cerebrum, cerebellum, spinal cord, and brain tissue samples from pigs SL44 and SL98 (infected with strain SW03) in Group 4 (Table 3).

### Pathological and histopathological findings

The gross pathological findings for Groups 1 and 2 (breeding wild boar), and Group 3 (breeding pigs) infected with strain YC11WB comprised swelling and congestion of the spleen and the submandibular and mesenteric lymph nodes, hemorrhage of the skin, and local petechial hemorrhage of the bladder, gallbladder, and kidney. Histopathological examination of the spleen and lymph nodes from breeding wild boar (W26, W28, NW32, and NW35) revealed various lesions, including depletion of long-lived plasma cells, reticuloepithelial cell hyperplasia, and endotheliosis (data not shown). Meningitis and perivascular cuffing were observed in the cerebrum of four breeding wild boar (W26, W28, NW32, and NW35) and two breeding pigs (R110 and R112) infected with strain YC11WB, but were much less prominent in the cerebrum of pigs infected with strain SW03.
Table 3. Viral RNA copy number in tissue samples from pigs infected with classical swine fever virus strains

| Group | Pig Number | Cs | Necropsy (DPI) | Organ |
|-------|------------|----|---------------|-------|
|       |            |    |               | To    | Lu | He | Sp | Li | ML | Ki | Il | Ce | LN | Ce | Cer | SC |
| 1     | W26        | YC | 17            | 2.17*  | 3.26 | 3.05 | 6.13 | 4.27 | 4.02 | 5.63 | 1.94 | 5.39 | 4.91 | 3.53 | 1.76 | 2.43 |
| 1     | W28        | YC | 18            | 6.87   | 3.75 | 4.64 | 3.89 | 6.03 | 3.52 | 4.57 | 4.11 | 5.87 | 2.04 | 1.85 | 1.49 | 2.36 |
| 2     | NW32       | YC | 18            | 3.67   | 4.06 | 3.22 | 6.38 | 5.54 | 2.63 | 4.02 | 4.98 | 5.81 | 6.26 | 3.44 | 1.62 | 2.73 |
| 2     | NW35       | YC | 21            | 4.29   | 3.77 | 3.64 | 2.10 | 3.61 | 1.54 | 5.85 | 4.45 | 1.06 | 4.87 | 3.29 | 2.47 | 2.64 |
| 3     | R110       | YC | 10            | 6.54   | 7.25 | 3.63 | 2.47 | 1.88 | 1.65 | 2.35 | 2.04 | 1.81 | 2.09 | 4.35 | 1.28 | 1.06 |
| 3     | R112       | YC | 13            | 5.05   | 5.69 | 3.48 | 4.81 | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| 4     | SL44       | SW | 21            | 4.28   | 2.03 | 1.92 | –   | –   | 1.84 | 4.42 | –   | 1.16 | 2.05 | –   | –   | 1.67 |
| 4     | SL98       | SW | 18            | 2.65   | 4.82 | 1.08 | 1.75 | –   | 2.27 | –   | 1.54 | 2.64 | –   | –   | –   | –   |
| 5     | W21        |    | 21            | –      | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| 5     | W22        |    | 21            | –      | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| 6     | L18        |    | 21            | –      | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |
| 6     | L22        |    | 21            | –      | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   | –   |

*Real-time polymerase chain reaction copy number. Cs, challenge strains; DPI, days post-infection; To, tonsil; Lu, lung; He, heart; Sp, spleen; Li, liver; ML, mesenteric lymph node; Ki, kidney; Il, ileum; Ce, cecum; LN, inguinal lymph node; Ce, cerebrum; Cer, cerebellum; SC, spinal cord; YC, YC11WB; SW, SW03; –, negative.

Animal survival rates

After challenge with strain YC11WB, the survival rate for Group 1 was 85.7% at 18 DPI, 57.1% at 20 DPI, 42.9% at 10 DPI, and 28.6% at 25 DPI (data not shown). The survival rate for Group 2 was 71.4% at 11 DPI, 57.1% at 13 DPI, 28.6% at 14 DPI, 14.3% at 15 DPI, and 0% at 16 DPI (data not shown). Strain YC11WB was fatal to breeding pigs at 11 to 16 DPI and to breeding wild boar at 18 to 25 DPI.

Discussion

In this study, we observed the aa differences between ABCD domain regions of the LOM vaccine strain and those of virulent CSFVs (SW03 and YC11WB) was particularly high (13.3%). These differences may have altered the topology of the mAb-binding sites in the D/A (mAbs 4F69 and 4F41) and B/C (mAbs 1A39 and 10A56) domains, thereby providing an explanation as to why these mAbs did not recognize the virulent CSFVs.

A previous study that examined the antibody responses of five overlapping synthetic peptides covering the antigenic domain B/C (aa 693–777) within envelope protein E2 showed that PV-BC1 (BC1: aa 693–716) elicited a potent protective response, whereas PV-BC3, PV-BC4, and PV-BC5 (BC3: aa 723–745; BC4: aa 741–760; BC5: aa 757–777) showed weaker activity [1]. Interestingly, three mAbs (7F2, 7F84, and 6F65) that bound to the B/C domain regions of strains LOM and SW03 in this study did not bind to that of YC11WB. The SW03 and YC11WB strains differed with respect to four aas: T692S, K720R, N723S, and I745M. Two epitope prediction programs (i.e., Emini Surface Accessibility Prediction and Bepipred Linear Epitope Prediction) predicted the presence of a strong epitope at aa positions 718–724 within the B/C domain, a region harboring two of the aa substitutions. The topography of the mAb-binding sites within E2 may be flexible, provided that conformational integrity is maintained [29]. A recent study that compared the sequences of the Spain-01 and Margarita CSFV strains identified several aa substitutions in the E2-B/C domains [22]. Substitutions D723S, N725G, T738V, G761R, and S777N were observed at sites reported to be under positive selection pressure, suggesting that these changes may have a role in viral escape from neutralizing antibodies [24].

Various studies examining disease symptoms and CSF pathology have been undertaken in several countries in an attempt to characterize the virulence and clinical effects of different CSFV strains in pigs [12,13,18,19,22,28]. The overall aim of those studies is the global elimination of CSF; indeed, the USA, Canada, and Japan, as well as members of the EU, have eradicated this disease successfully. However, sporadic outbreaks of CSF that occurred in domestic pigs in Europe were linked to indirect or direct contact with wild boar [4]. Indeed, it is estimated that 59% of primary CSF outbreaks in Germany over a period of 10 years were caused by contact between wild boar and domestic pigs [6,7].

Korea has experienced many CSF outbreaks on breeding pig farms after the genotype 2 shift occurred in 2002: 13 in 2002, 72 in 2003, nine in 2004, five in 2005, two in 2006, five in 2007, seven in 2008, two in 2009, and one in 2013. All were attributed to strain SW03. In the present study, the observed mortality rate for SW03 during the 22 day period was 50%, and the
histopathological lesions observed in the organ tissue samples were less severe than those observed after infection by UC11WB. The UC11WB isolate produced more serious symptoms and higher mortality in breeding pigs than those from the SW03 isolate. However, anti-CSFV antibodies induced by vaccination would protect against all genotypes as the vaccine is derived from a single serotype.

Therefore, active disease surveillance and CSF vaccination should be undertaken regularly on breeding farms located at the base of mountains or near forest areas in Korea to prevent spillover transmission of CSF from wild boar to domestic pigs. In addition, farms should be surrounded by perimeter fences to prevent direct contact between wild and domestic animals.

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. Bautista MJ, Ruiz-Villamor E, Salguero FJ, Sánchez-Cordón PJ, Carrasco L, Gómez-Villamandos JC. Early platelet aggregation as a cause of thrombocytopenia in classical swine fever. Vet Pathol 2002, 39, 84-91.
2. Berman HM, Westbrook J, Feng Z, Bhat TN, Weissig H, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000, 28, 235-242.
3. Blacksell SD, Khounsy S, Van Aken D, Gleeson LJ, Westbury HA. Comparative susceptibility of indigenous and improved pig breeds to classical swine fever virus infection: practical and epidemiological implications in a subsistence-based, developing country setting. Trop Anim Health Prod 2006, 38, 467-474.
4. Chang CY, Huang CC, Lin YJ, Deng MC, Chen HC, Tsai CH, Chang WM, Wang FI. Antigenic domains analysis of classical swine fever virus E2 glycoprotein by mutagenesis and conformation-dependent monoclonal antibodies. Virus Res 2010, 149, 183-189.
5. Chang CY, Huang CC, Lin YJ, Deng MC, Tsai CH, Chang WM, Wang FI. Identification of antigen-specific residues on E2 glycoprotein of classical swine fever virus. Virus Res 2010, 152, 65-72.
6. Das A, Beckham TR, McIntosh MT. Comparison of methods for improved RNA extraction from blood for early detection of classical swine fever virus by real-time reverse transcription polymerase chain reaction. J Vet Diagn Invest 2011, 23, 727-735.
7. Depner KR, Hinrichs U, Bickhardt K, Greiser-Wilke I, Pohlenz J, Moennig V, Liess B. Influence of breed-related factors on the course of classical swine fever virus infection. Vet Rec 1997, 140, 506-507.
8. Dong XN, Qi Y, Ying J, Chen X, Chen YH. Candidate peptide-vaccine induced potent protection against CSFV and identified a principal sequential neutralizing determinant on E2. Vaccine 2006, 24, 426-234.
9. Eminii EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 1985, 55, 836-839.
10. Everett H, Salguero FJ, Graham SP, Haines F, Johns H, Clifford D, Nunez A, La Rocca SA, Parsharianon S, Steinbach F, Drew T, Crooke H. Characterisation of experimental infections in domestic pigs with genotype 2.1 and 3.3 isolates of classical swine fever virus. Vet Microbiol 2010, 142, 26-33.
11. Floegel-Niesmann G, Blome S, Gers-Düllmier H, Bunzenthal C, Moennig V. Virulence of classical swine fever virus isolates from Europe and other areas during 1996 until 2007. Vet Microbiol 2009, 139, 165-169.
12. Floegel-Niesmann G, Bunzenthal C, Fischer S, Moennig V. Virulence of recent and former classical swine fever isolates evaluated by their clinical and pathological signs. J Vet Med B Infect Dis Vet Public Health 2003, 50, 214-220.
13. Fritzemeier J, Teuffert J, Greiser-Wilke I, Staubach C, Schützer H, Moennig V. Epidemiology of classical swine fever in Germany in the 1990s. Vet Microbiol 2000, 77, 29-41.
14. Gomez-Villamandos JC, Salguero FJ, Ruiz-Villamor E, Sánchez-Cordón PJ, Bautista MJ, Sierra MA. Classical swine fever: pathology of bone marrow. Vet Pathol 2003, 40, 157-163.
15. Jeoung HY, Lim JA, Lim SI, Kim JJ, Song JY, Hyun BH, Kim YK, An DJ. Complete genome sequence of classical swine fever virus isolated from wild boar in South Korea. Genome Announc 2013, 1, e00147-13.
16. Jespersen MC, Peters B, Nielsen M, Marcattili P, BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 2017. Epub ahead of print. doi: 10.1093/nar/gkx346.
17. Kaden V, Lange E, Polster U, Klopfeisch R, Teifke JP. Studies on the virulence of two field isolates of the classical swine fever genotype 2.3 Rostock in wild boars of different age groups. J Vet Med B Infect Dis Vet Public Health 2004, 51, 202-208.
18. Laddomada A. Incidence and control of CSF in wild boar in Europe. Vet Microbiol 2000, 73, 121-130.
19. Lindenbach BD, Rice CM. Flaviviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE (eds.). Fundamental Virology. 4th ed. pp. 589-639, Lippincott Williams & Wilkins, Philadelphia, 2001.
20. Meyers G, Rümenapf T, Thiel HJ. Molecular cloning and nucleotide sequence of the genome of hog cholera virus. Virology 1989, 171, 555-567.
21. Mintiens K, Verloo D, Venet E, Laeves H, Dufey J, Dewulf J, Boelaert F, Kerkhofs P, Koenen F. Estimating the probability of freedom of classical swine fever virus of the East-Belgium wild-boar population. Prev Vet Med 2005, 70, 211-222.
22. Mittelholzer C, Moser C, Tratschin JD, Hofmann MA. Analysis of classical swine fever virus replication kinetics allows differentiation of highly virulent from avirulent strains. Vet Microbiol 2000, 74, 293-308.

23. Morten Källberg, Haipeng Wang, Sheng Wang, Jian Peng, Zhiyong Wang, Hui Lu, Jinbo Xu. Template-based protein structure modeling using the RaptorX web server. Nat Protoc 2012, 7, 1511-1522.

24. Nielsen J, Lohse L, Rasmussen TB, Uttenthal A. Classical swine fever in 6- and 11-week-old pigs: haematological and immunological parameters are modulated in pigs with mild clinical disease. Vet Immunol Immunopathol 2010, 138, 159-173.

25. Pérez LJ, Diaz de Arce H, Perera CL, Rosell R, Frías MT, Percedo MI, Tarradas J, Domínguez P, Núñez JJ, Ganges L. Positive selection pressure on the B/C domains of the E2-gene of classical swine fever virus in endemic areas under C-strain vaccination. Infect Genet Evol 2012, 12, 1405-1412.

26. Summerfield A, Knöting SM, McCullough KC. Lymphocyte apoptosis during classical swine fever: implication of activation-induced cell death. J Virol 1998, 72, 1853-1861.

27. Summerfield A, McNeeley F, Walker I, Allan G, Knoetig SM, McCullough KC. Depletion of CD4+ and CD8high T-cells before the onset of viraemia during classical swine fever. Vet Immunol Immunopathol 2001, 78, 3-19.

28. Wee SH, Park CK, Jeong JM, Kim CH, Hwang JJ, Kim SJ, Yoon H, Lee ES, Nam HM, Park JY, Moon OK. Outbreaks of classical swine fever in the Republic of Korea in 2003. Vet Rec 2005, 157, 113-115.

29. Weiland E, Stark R, Haas B, Rümenapf T, Meyers G, Thiel HJ. Pestivirus glycoprotein which induces neutralizing antibodies forms part of a disulfide-linked heterodimer. J Virol 1990, 64, 3563-3569.

30. Wensvoort G. Topographical and functional mapping of epitopes on hog cholera virus with monoclonal antibodies. J Virol 1989, 70, 2865-2876.

31. Wensvoort G, Boonstra J, Bodzinga BG. Immunoaffinity purification and characterization of the envelope protein E1 of hog cholera virus. J Gen Virol 1990, 71, 531-540.

32. Wensvoort G, Terpstra C, Boonstra J, Bloemraad M, van Zaane D. Production of monoclonal antibodies against swine fever virus and their use in laboratory diagnosis. Vet Microbiol 1986, 12, 101-108.