Evolution and molecular epidemiology of classical swine fever virus during a multi-annual outbreak amongst European wild boar

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Classical swine fever is a viral disease of pigs that carries tremendous socio-economic impact. In outbreak situations, genetic typing is carried out for the purpose of molecular epidemiology in both domestic pigs and wild boar. These analyses are usually based on harmonized partial sequences. However, for high-resolution analyses towards the understanding of genetic variability and virus evolution, full-genome sequences are more appropriate. In this study, a unique set of representative virus strains was investigated that was collected during an outbreak in French free-ranging wild boar in the Vosges-du-Nord mountains between 2003 and 2007. Comparative sequence and evolutionary analyses of the nearly full-length sequences showed only slow evolution of classical swine fever virus strains over the years and no impact of vaccination on mutation rates. However, substitution rates varied amongst protein genes; furthermore, a spatial and temporal pattern could be observed whereby two separate clusters were formed that coincided with physical barriers.

Classical swine fever virus (CSFV) is one of the most important viral pathogens capable of causing disastrous economic losses in pig industries worldwide (Edwards et al., 2000). Endemically infected wild boar populations can serve as a natural reservoir (Artois et al., 2002; Fritzemeier et al., 2000; Laddomada, 2000; Leifer et al., 2010), and thus contribute to virus persistence and spread. CSFV belongs to the family Flaviviridae, genus Pestivirus, and is characterized by a positive-sense ssRNA genome that contains 5'- and 3'-terminal NTRs that enclose one ORF of ~12 300 nt (Meyers & Thiel, 1996). This ORF encodes one polyprotein (Meyers & Thiel, 1996) that is processed into four structural proteins (C, E1, E2, and E3) and the non-structural proteins Npro, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Rümenapf et al., 1993; Tautz et al., 1997; Thiel et al., 1991). CSFV strains can be assigned to three genotypes with three to four subtypes (Postel et al., 2012, 2013). The distributions of genotypes show distinct geographical patterns (Beer et al., 2015; Paton et al., 2000), and genetic typing and phylogenetic analyses are valuable tools to elucidate the molecular epidemiology of outbreaks. To date, routine CSFV genetic typing and molecular epidemiological studies are based on a 150 nt fragment of the 5'-NTR and a 190 nt fragment of the E2 protein gene (Greiser-Wilke et al., 2000, 2006; Paton et al., 2000). To improve CSFV molecular epidemiology, the use of full-length E2 encoding sequences was recently recommended by the EU and OIE Reference Laboratory for Classical Swine Fever (Postel et al., 2012). However, full-genome sequences are best suited for evolutionary studies, and reflect more accurately the genetic variability and substitution rate across different genotypes (Djikeng & Spiro, 2009). Up to now, exact information on CSFV mutation rates and evolutionary characteristics has been scarce and based on datasets collected from sequence databases (Ji et al., 2014; Zhang et al., 2013). Thus, data are especially missing for long-term evolution under natural conditions. The lack of data is partly due to the fact that collection from domestic pigs is hampered by the immediate culling of CSFV-infected...
herds. Additionally, metadata are often missing in endemically infected countries.

In the present study, we used a unique collection of 11 CSFV genotype 2.3-positive spleen samples from a long-term outbreak of classical swine fever amongst wild boar in the Bas-Rhin and Moselle districts within the Vosges-du-Nord mountains, France, that had been used for initial molecular epidemiology (Simon et al., 2013).

In order to conduct in-detail evolutionary analyses, we used next-generation sequencing to generate nearly full-length genomes of these virus strains that were representative of different variants from several geographical locations during the outbreak period between 2003 and 2007 in Bas-Rhin (Fig. 1a, Table S1, available in the online Supplementary Material). To avoid cell culture-influenced substitutions, sequences should be derived directly from the diagnostic specimen. Unfortunately, direct sequencing was not successful in the present case as several samples had very low viral loads. Thus, a compromise was needed, and a conventional reverse transcription (RT)-PCR protocol was designed that covered the complete ORF and parts of the 5′- and 3′-NTRs in overlapping amplicon fragments. RNA was isolated using TRIzol reagent (Invitrogen) combined with the manufacturer's protocols. The reads obtained after sequencing were assembled using the 454 assembler software Newbler version 2.6 (Roche).

Nearly full-length CSFV sequences (12,104 nt) were obtained from all 11 wild boar spleen samples, and were analysed comparatively with BioEdit version 7.0.9.0. (Hall, 1999) and MEGA5 (Tamura et al., 2011). Both phylogenetic and spatial and temporal evolutionary analyses were conducted with both full-length sequences and datasets for each coding protein gene, as well as the partial 5′- and 3′-NTRs. Additionally, 23 recently published partial sequences (5′-NTR/E2/NSSB) obtained from the same outbreak in Bas-Rhin between 2003 and 2007 (Simon et al., 2013) were added to the sequences obtained in our study, aligned and concatenated.

Phylogenetic analyses were performed with neighbour-joining methods and the Tamura–Nei (TN) parameter as estimated with model testing in MEGA5 (Tamura et al., 2011). Substitution rates and divergence times were estimated using a Markov chain Monte Carlo (MCMC) approach implemented in BEAST version 1.8.0 (Drummond et al., 2012).

The evolutionary rates were estimated with the Hasegawa–Kishino–Yano (HKY) substitution model, and additionally with the respective substitution model estimated with model testing (data not shown) using an uncorrelated log-normal relaxed clock model and an uncorrelated log-normal distribution. Evolutionary rates were estimated based on the exact sampling dates (day, month and year) and on the year only. The MCMC chain had a total length of $1 \times 10^9$ for each of the genome parts and was sampled every $1 \times 10^3$ generations. Data were analysed using Tracer version 1.5 (http://beast.bio.ed.ac.uk/Tracer), and the maximum clade credibility trees for the 11 full genomes and the 34 concatenated sequences were calculated with TreeAnnotator version 1.8.0 (http://beast.bio.ed.ac.uk/TreeAnnotator). Trees were visualized with FigTree version 1.4.0 (http://beast.bio.ed.ac.uk/figtree). Additionally, metadata on oral emergency vaccination were available to investigate potential selective pressures through control measures. In detail, oral emergency vaccination was implemented in the first infected area starting in August 2004 (Fig. 1a), and the area was enlarged in January 2005 to cover all forested areas at risk (Pol et al., 2008; Rossi et al., 2010). From 2005 to 2010, three vaccination campaigns were applied per year and the last positive-virus isolation case was recorded in May 2007 (La Petite Pierre municipality). Preventive vaccination was maintained until June 2010 (Calenge & Rossi, 2014).

In general, sequence identity of all French viruses during the outbreak in the Vosges-du-Nord mountains was very high, indicating that during the outbreak the CSFV viruses were evolving slowly, as described previously by Simon et al. (2013). Comparative pairwise sequence analysis revealed that the sequence identity between the French CSFV sequences ranged from 99.3% for CSFV 1_05/2003 and CSFV 11_01/2005 to 99.8% for CSFV 1_05/2003 and CSFV 2_11/2003 (Fig. S1). However, a clear increase in unique nucleotide substitutions (substitutions that were not previously observed at a given position in the genomes of the strains investigated in this study) could be observed from year to year (Table 1). Most unique nucleotide substitutions in relation to the sequence length were detected in the structural E1 protein gene with nine out of 36 total substitutions (1.5% across the sequence length of the E1), of which two were non-synonymous (1.0% unique amino acid substitutions in E1) (Table 1), and the fewest unique nucleotide substitutions were observed in the Ems protein gene (three unique substitutions of seven total substitutions across the sequence length; 0.44%, Table 1). The corresponding evolutionary substitution rate was likewise the highest within the structural protein genes, with $3.8 \times 10^{-3}$ substitutions site$^{-1}$ year$^{-1}$ (Table 1). Fewer unique nucleotide substitutions in relation to the sequence length were observed in the most important immunogenic E2 protein gene, and the corresponding estimated substitution rate was accordingly also lower (Table 1). Compared with other studies of CSFV type 2.3 strains from roughly the same time (Leifer et al., 2010), fewer substitutions for initial molecular epidemiology (Simon et al., 2013).
Fig. 1. (a) Map displaying the geographical locations of the respective CSFV-positive wild boar detected in Bas-Rhin between 2002 and 2007. Red dots, CSFV variants from 2003 and 2004 in the north-eastern (NE) region of the National Park for which full genome sequences were obtained in this study; blue dots, CSFV variants from 2004 to 2007 in the south-western (SW) region of the National Park for which full genome sequences were obtained in this study. Red triangles, CSFV variants in the north-eastern region of the National Park for which partial sequencing was conducted by Simon et al. (2013); blue triangles, CSFV variants in the south-western region of the National Park for which partial sequencing was conducted by Simon et al. (2013). (b) Unrooted neighbour-joining tree constructed using the TN parameter as implemented in MEGA5 (Tamura et al., 2011). Numbers at nodes indicate percentage of 1000 bootstrap replicates (values <50 are not shown). Bar, nucleotide substitutions per site. Red branches, north-eastern cluster; blue branches, south-western cluster.
**Table 1.** Absolute values of total and unique nucleotides as well as amino acid substitutions among the CSFV sequences obtained in this study

Numbers of substitutions were calculated for each protein gene and for the partial 5'– and 3'–NTRs. Relative values of substitutions (in relation to the sequence length of each gene), as well as estimated substitution rates (substitutions site⁻¹ year⁻¹) for the nucleotide sequences, are displayed.

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*NA, Not applicable.*

*HKY, Hasegawa–Kishino–Yano; JC, Jukes–Cantor; K2, Kimura two-parameter; TN, Tamura–Nei; HPD, highest posterior density.*
were observed in the E2 protein gene. This discrepancy could have resulted from the limited number of virus variants available from the reported German outbreak. However, in general, the two main structural surface glycoproteins, E1 and E2, showed the highest number of nucleotides, as well as amino acid substitutions, and the highest substitution rates within the structural proteins. This is not surprising, as these viral surface proteins are more prone to selective pressures by the host and thus need to have a higher mutation rate in order to evade the host’s immune response. Comparing all genome regions separately, the substitution rates ranged from 1.1 \times 10^{-3} substitutions site^{-1} year^{-1} in protein gene E^ms to 8.6 \times 10^{-3} substitutions site^{-1} year^{-1} in 3’-NTR (Table 1). No substitutions were observed in the p7 protein gene, and thus a further evolutionary estimation of a substitution rate was not possible.

The substitution rate estimated for the complete genome was 1.5 \times 10^{-3} substitutions site^{-1} year^{-1} when using the exact sampling dates (Table 1) and 9.9 \times 10^{-4} substitutions site^{-1} year^{-1} when using only the year of sampling. Estimation of the substitution rate for the 34 concatenated partial sequences resulted in 2.4 \times 10^{-3} substitutions site^{-1} year^{-1}. Similar or even identical evolutionary rates for all protein genes, the partial NTRs and the full genome were obtained when using the respective evolutionary substitution model estimated with model testing (Table 1). Typically, substitution rates of RNA viruses obtained from in silico analyses using partial genome fragments are estimated to be \sim 1.0 \times 10^{-3} substitutions site^{-1} year^{-1}; however, variations in substitution rates can be observed in different virus species (Jenkins et al., 2002). When comparing the estimated substitution rates obtained in this study to in silico analyses from CSFV sequences from China, the E2-based substitution rates obtained from Chinese CSFV strains are higher than those observed across the full-genome sequence in the presented study (Ji et al., 2014; Zhang et al., 2013). Similar evolutionary investigations of partial sequences revealed, for example, a substitution rate of 3.0 \times 10^{-3} substitutions site^{-1} year^{-1} (Ji et al., 2014; Zhang et al., 2013) for CSFV type 2 strains, which is similar to the substitution rate estimated in the presented study for the E2 protein gene (Table 1; 2.4 \times 10^{-3} substitutions site^{-1} year^{-1}). Another study from Chinese CSFV type 2 strains estimated \sim 1.8 \times 10^{-3} substitutions site^{-1} year^{-1} for the E2 protein gene and \sim 1.6 \times 10^{-3} substitutions site^{-1} year^{-1} for the concatenated genome without the NTRs (Ji et al., 2014), which is identical to the substitution rate for the full-genome sequences obtained in this study when estimated with the TN parameter (Table 1). These results suggest that BEAST analyses applied to only a few CSFV variants across a relatively short time frame within a geographically restricted area estimate results comparable to those obtained from large datasets. The evolutionary rates obtained were comparable amongst the models used for assessment, i.e. HKY or the model calculated by model testing. Therefore, reliability of the analyses is ensured.

These results indicate that evolutionary analyses using BEAST for the estimation of substitution rates reflect the reality, at least for the respective protein genes and the NTRs. However, the differences in the substitution rates between the full-genome sequences and the partial sequences also lead to the conclusion that a precise and unbiased estimation of evolutionary substitution rates for the whole virus per se is only possible when using full genomes rather than using partial genome fragments, as the estimated substitution rates could then be misleading.

Both phylogenetic and evolutionary analysis revealed two spatial and temporal clusters (Fig. 1a, b). The observed clusters were separated by a major road, a parallel railway line and a small river (Fig. 1a). Such physical borders with no fences in between were previously shown within the same area to temporarily slow the speed of CSFV spread, but not to prevent virus invasion, possibly due to the continuity of forested areas facilitating the crossing of terrestrial wildlife (Rossi et al., 2010). Therefore, we assume that few infected wild boar crossed this imperfect barrier, generating a particular strain selection that was based on the limitation of the host’s movements rather than on selection pressure on the virus itself. The split in the phylogenetic tree is supported by a strong bootstrap value of 100 (Fig. 1b), and common nucleotide substitutions in CSFV 3 and CSFV 4 and common nucleotide substitutions in the sequences of CSFV 5 and CSFV 6 (Fig. S1). Based on BEAST analyses, the estimation of the most recent common ancestor of the first case in 2003 dates back to March of that year (Fig. 2). Despite the above-mentioned overall stability of the virus strains, a relatively high number of substitutions could be observed between the 2004 variants in the north-eastern region and the 2004–2007 variants in the south-western region, indicating probably that between 2003 and 2004, different CSFV variants circulated between both regions in the Vosges-du-Nord mountains. A similar spatial pattern was observed when analysing the concatenated 34 partial sequences (Simon et al., 2013; see also Fig. S2), indicating that partial sequences are in most cases sufficient for rapid genetic typing.

As vaccination started in August 2004, we might have expected significant vaccine-induced evolutionary selective pressures. This hypothesis is, however, not supported by our results, as evolution did not appear to be faster during the times of intensive vaccination (August 2004 to May 2007). Investigations of the relevant immunogenic regions (known B- and T-cell epitopes) of the E2 protein gene revealed only one non-synonymous substitution in the proposed T-cell epitope VCTAGSFKVTALNVV of CSFV 4 from 2004, and a synonymous substitution in the T-cell epitope DYRYAISSTNEIGLLG (Armengol et al., 2002) in CSFV 9 from 2006. The observed substitutions did not occur in any of the other isolates, indicating that the substitutions were most likely not induced by vaccination, which had started by late August 2004.

To the best of our knowledge, this is the first report on evolutionary investigations on CSFV during a long-term
outbreak in wild boar under natural conditions where exact metadata were available. Whilst partial sequences are generally sufficient for rapid genetic typing and for first insights into molecular epidemiology, the spatio-temporal reality is only reflected using full-length genomes and related metadata. Thus, those approaches should be implemented in order to obtain a better understanding of the evolutionary history, the long-term persistence or re-emerging patterns of classical swine fever outbreaks in wild boar, and the impact of the landscape on further spreading. Such investigations rely on the long-term collection of virus strains and international collaborations between neighbouring countries.

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References


