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# Vaccination influences the evolution of classical swine fever virus

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ABSTRACT

Classical swine fever is a serious, economically damaging disease caused by classical swine fever virus (CSFV). The CSFV is composed of two clades, according to phylogenetic estimates. Attenuated live vaccine such as HCLV, has been widely used to protect pigs from CSFV, but the influence of vaccination on the evolution of CSFV has not been studied. We conducted a systemic analysis of the impact of vaccination on the evolution of CSFV by comparing vaccine-related and non-vaccine-related CSFV groups. We found that vaccination may affect strain diversity and immune escape through recombination and point mutation. We also found that vaccination may influence the population dynamics, evolutionary rate and adaptive evolution of classical swine fever virus. Our evidence suggests that the vaccination might also change host adaptation through influencing codon usage of the virus in swine. These findings suggest that it is necessary to avoid excessive use of CSFV attenuated vaccines.



## 1. Introduction

Classical swine fever virus (CSFV) is a single stranded, non-segmented, positive-sense RNA virus that belongs to the family *Flaviviridae*, genus *Pestivirus* (Francki et al., 1991). This lipid-enveloped RNA virus infects domestic and wild pigs worldwide, and causes major losses in stock farming. CSFV infection is associated with disseminated intravascular coagulation, thrombocytopenia and immunosuppression (Yin and Liu, 1997). The CSFV genome is approximately 12,300 nucleotides in length and comprises a long open reading frame (ORF) flanked by 5′ and 3′untranslated regions (UTRs). The ORF is translated to a primary polyprotein and then subsequently processed into 12 mature proteins by cellular and viral proteases, including eight nonstructural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) and four structural proteins (C, Erns, E1, E2; (Rice, 1996; Thiel et al., 1991).

Classical swine fever (CSF) was first recognized in Tennessee, USA in 1810 (Agriculture, 2010). It was described in France in 1822 (Cole, 1962) and within a few years it was found all over the world, including the UK in 1864 (Agriculture, 2010), Japan in 1888 (Edwards et al., 2000), the Caribbean in 1930 (Cuba; (Edwards et al., 2000), and China in 1925 (Tu et al., 2001). Three CSFV genotypes have been recognized in 36 countries and are still in circulation (Food and Agriculture, 1990; Paton et al., 2000). Genotype1 consists of lentogenic and velogenic viruses and are mainly found in CSFV outbreaks in China (Zhou, 1980). The other two genotypes are made up of virulent viruses and exist in other parts of the world.

Vaccination has been widely used to protect pigs from CSFV. From 1940 to 1949, two attenuated strains (the ROVAC strain from the US and the SFA strain from England) were obtained by repeatedly adapting viruses to rabbits (Baker, 1946; Koprowski et al., 1946). In 1954, Lee and colleagues continued to adapt the strain ROVAC (250-generation passages) in rabbits and obtained a lapinized strain (LPC) after 800 generations (Lin Tc Fau - Shieh et al., 1974; Lin and Lee, 1981). In 1955, a stable lapinized strain was obtained by passaging the virulent Shimen strain in rabbits. This strain was named the Chinese vaccine strain (C-strain) or the hog cholera lapinized virus (HCLV) and has been widely used in mainland China since 1957. The HCLV strain has played a global role in controlling CSFV epidemic in domestic pigs (TC, 1980; Yin and Liu, 1997; Zhou, 1980). HCLV has been introduced to many other countries because it is believed to be safer and more effective than the other commercial vaccines (e.g. American ROVAC and British SFA; (Bognar K, 1963; Olah and Palatka, 1967). Many commercial vaccine strains have been derived from HCLV (TC, 1980), such as Pestiffa (France), SUVAC (Hungary), Lapest (Poland), SuiferinC (former East Germany), ЛК (former USSR), VADIMUN (USA), and Riems (Germany). By passage the virulent viruses in certain cells under low temperatures (29–30 °C), investigators also recovered some attenuated strains, for instance a Japanese guinea-pig exaltation-negative strain (GPE−), derived from the virulent strain ALD (Sasahara, 1970; Sasahara et al., 1969) and a French cell culture adapted strain Thiverval, derived from virulent strain Alfort (Aynaud et al., 1970; Launais et al., 1978; Lunais M et al., 1974;

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Lunais M, 1977; LunaisM, 1972). Currently, the routinely used vaccines are all live attenuated vaccines including C-strain, GPE−, Thiverval and their derivative strains (Van Oirschot, 2003). All of these live attenuated vaccines belong to the genotype I group.

The evolutionary impact of the CSFV vaccination has not been studied although it has successfully protected pigs. In this study, we investigate the role of vaccination in shaping the evolution of CSFV using available isolates. In particular, we focused on the evolutionary effects on HCLV. Our results demonstrate that vaccination can potentially result in novel CSFV forms through recombination between vaccine strains and wild strains and we provide evidence that the vaccine-related group experiences different mutation rates, population sizes, selection profiles and codon usage bias than the non-vaccine-related CSFV strains.

## 2. Results

### 2.1. Phylogenetic classification and origin of CSFV

We recovered two monophyletic CSFV groups in the E2 gene tree (Figs. 2 and S1). One group included all of the attenuated vaccine strains, named the vaccine-related group, and the other group is named the non-vaccine-related group. The BEAST analysis estimates that CSFV may have appeared in China in the 1920s (95% HPD: 1856–1970) and the two CSFV groups may have diverged in the early 1960s (Fig. 2). These results agree with observations of when CSFV originated in China (Tu et al., 2001; Yin and Liu, 1997; Zhou, 1980).

### 2.2. Attenuated CSFV vaccine can potentially influence CSFV evolution through recombination with wild virus

Homologous recombination is an important evolutionary force and previous studies have found that homologous recombination can occur in CSFV (He et al., 2007). To know whether CSFV vaccines can recombine with circulating strains, we reanalyzed all available CSFV genomes in RDP4 with nine different algorithms (Martin et al., 2010). Five mosaic viruses were detected (7.8% of the 64 complete genomes) with two putative parents and $p$ value less than $10^{-5}$ in each putative breakpoint. The five recombinants were HCLV strain, strain 39, SWH, Heb 52010 and strain ALD (Figs. 3 and S2–S5). Interestingly, the isolate associated with HCLV has a

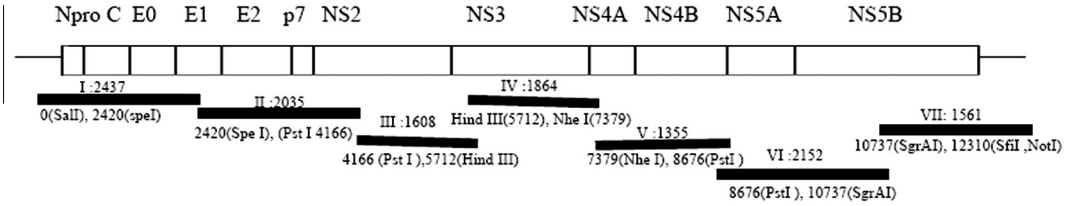


**Fig. 1.** Schematic representation of cDNA clones to determine the nucleotide sequence of the C strain of CSFV.

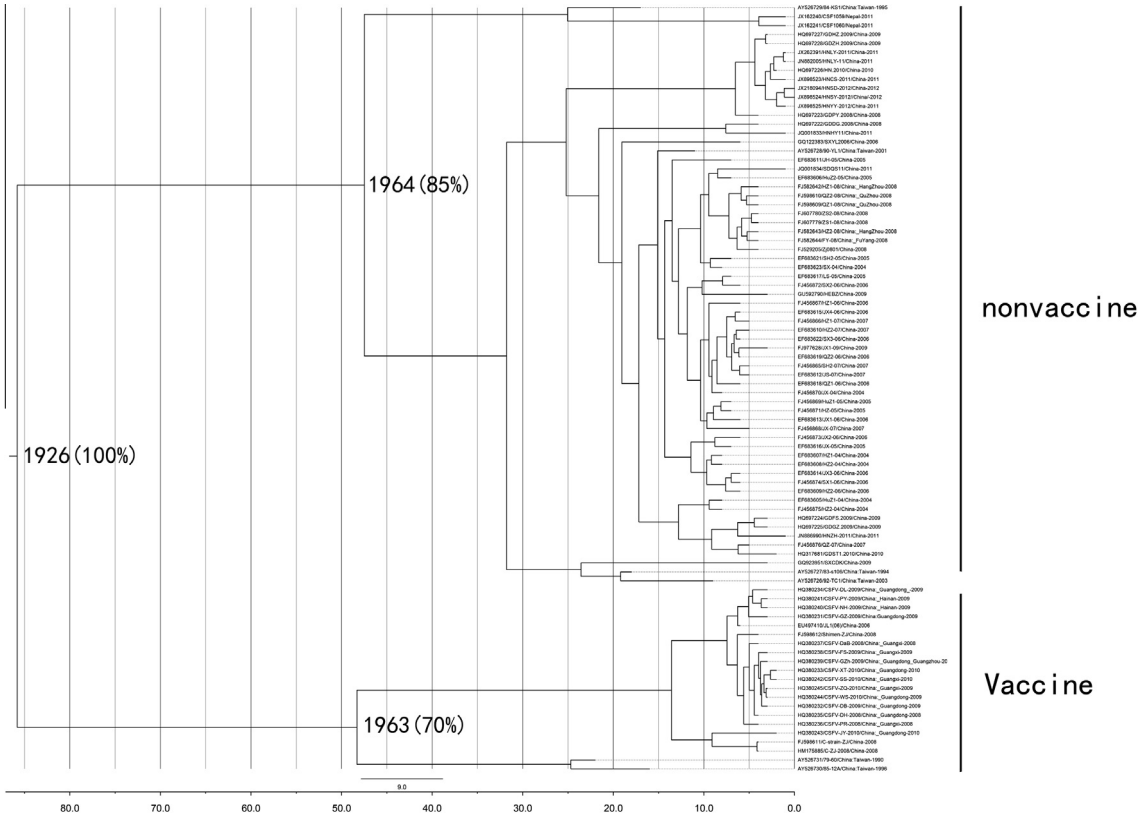


**Fig. 2.** An MCC tree of the E2 gene of CSFV from China. The trees were scaled to time using the collection ages of some CSFV samples, the GTR + G6 substitution model and an uncorrelated exponential molecular clock. Median tMRCAs are shown for selected nodes with the posterior probability.