Evolutionary Rate of E2 Genes of Classical Swine Fever Virus in China

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ABSTRACT
Classical swine fever (CSF) is caused by classical swine fever virus (CSFV), a member of the genus Pestivirus of the family Flaviviridae, and engender important economic ramifications. Our previous study reported that both CSFV Group 1 and Group 2 were both contributed to the epidemic of CSF in mainland China, and showed the trend of switch from Group 1 to Group 2. In order to investigate the relationship between epidemiological trend and evolutionary rate of two Groups, the E2 glycoprotein gene (located in 2508-2697) of 68 CSFVs isolated from mainland China during 1982-2009 were aligned, and Bayesian Markov Chain Monte Carlo (MCMC) analysis was performed. The results indicated that the mean evolutionary rate of Group 2 (3.6861×10⁻³ substitutions per site per year (subs/site/year)) evolved much faster than Group 1 (4.9852×10⁻⁴ subs/site/year). We presumed that the differences in evolutionary rates of two groups likely implied that Group 2 possessed higher mutation rate and experienced higher selection pressure, however the real mechanism for the diversity in the evolutionary rate requires further investigation.

Key words: classical swine fever virus, envelope glycoprotein E2, evolutionary rate, MCMC, selection pressure

INTRODUCTION
Classical swine fever (CSF, alias hog cholera) is a serious infectious disease of pigs, which is notifiable to the World Organization for Animal Health (OIE) and to the European Union (EU) (1). CSF is a devastating disease that poses one of the greatest risks to the swine industry and cause great economic loss in China, with continuing sporadic outbreaks in several different provinces of the mainland (2). Classical swine fever virus (CSFV), bovine viral diarrhea virus type I and type II (BVDV-I and BVDV-II) and border disease virus (BDV) belong to the Pestivirus genus of the Flaviviridae family (3). CSFV is a small (40-60 nm in diameter) enveloped positive-stranded RNA virus and contains a genome about 12.3 kb. CSFV genomes have a large open reading frame (ORF) flanked by highly conserved 5′ non-translational region (5′-NTR) and 3′-NTR, and ORF codes for a unique polyprotein of about 3898 amino acids (Npro-C-Ecapsid-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B) (4). The polyprotein gives rise to autoprotease (Npro), four structural proteins (C, Ecapsid, E1 and E2), and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) upon processing by cellular and viral proteases (5). E2 gene, together with NS5B and 5′-NTR were used for evolutionary analysis and resulted in the same resolution (Fig 1) (2, 6, 7).

Envelope protein E2 is the major envelope glycoprotein exposed on the outer surface of the virion and represents an important target for induction of the immune response during infection (8). There are four antigenic domains in the N-terminal half of E2, (A, B, C and D), with three subdomains (A1, A2 and A3) in domain A. Domains B and C as well as subdomain A1 are neutralizing but only subdomain A1 is conserved (9). The 190 nt in this variable region of N-terminal is extensively used for evolutionary analysis (7, 10).

Despite intense immunization and even eradication ef-
forts, the total number of outbreaks reported by Chinese veterinary laboratories has increased during the last twenty years (11, 12). Phylogenetic analysis indicates that CSFV could be classified into three Groups (Group 1, 2 and 3) (13). Our previous study reported that the Chinese traditional isolates mainly fall into groups one or two: Group 1 comprises mainly the modified live vaccines and many highly virulent strains and group 2 mainly recent and moderately virulent isolates. Both CSFV Group 1 and Group 2 have contributed to the epidemic of CSF in mainland China, with a trend of switch from Group 1 to Group 2 (2). The epidemiology of CSFV is important and there is a need to investigate its epidemiological status and the relationship between epidemiological trend and evolutionary rate of two groups. In particular the evolutionary rate between two genotypes of CSFVs remains elusive. Thus an understanding the evolutionary rate of the glycoprotein E2 of CSFV could possibly provide clues for the epidemiological characterization, as well as reveal the possible evolutionary strategies that different genotypes of CSFVs have adopted.

MATERIALS AND METHODS

Virus sequences
The 190 nt of E2 sequences (located in 2518-2707) of 68 representative CSFV isolates were retrieved form GeneBank website (http://www.ncbi.nlm.nih.gov/), EMBL website (http://www.ebi.ac.uk/embl/) and EU reference laboratory for CSFV database in Hannover (http://viro08.tiho-hannover.de/eg/csf/startCSF.cgi), respectively. All CSFV isolate datasets are listed in Table 1.

Estimation of evolutionary rate
Evolutionary rate of E2 genes of classical swine fever virus were represented with nucleic acid substitution rates and were analyzed independently. The E2 gene sequences of CSFV isolates from China were compiled and aligned using Clustal X software (version 1.83) (14), and then the best-fitting model of nucleotide substitution for each dataset was determined using jModeltest (version 3.7) (15). Firstly, the best-fitting models were determined as Hasegawa-Kishino-Yano (HKY) model for Group 1 and the General Time Reversible with Gamma (GTR+G) model for Group 2, respectively (Table 2). Secondly, the variable-rate relaxed molecular clock models were determined best-fitting to Group 1 and Group 2. Thirdly, Bayesian Markov Chain Monte Carlo approach (MCMC, the BEAST package, version 1.5.1) (16) was used to estimate the viral substation rates. The final calculated results were viewed by Tracer software (in Tracer 1.4, http://beast.bio.ed.ac.uk/Tracer). Mean values are expressed as well as 95% high probability density intervals (HPD).
The respective evolutionary rate of Group 1 and Group 2 are shown in Table 3, and mean nucleotide substitution rate with 95% HPD are displayed in Fig 2. Our previous study reported that both CSFV Group 1 and Group 2 were both contributed to the epidemic of CSF in mainland China (2). However, it is noteworthy that Group 2 of CSFV’s, with a evolutionary rate of 3.6861×10⁻³ subs/site/year (95% HPD 2.0816×10⁻³-5.5134×10⁻³), was approximately seven times that of Group 1 with a mean substitution rate of 4.9852×10⁻⁴ subs/site/year (95% HPD 3.1189×10⁻⁵-1.1018×10⁻³). Compared with Group 2, 95% HPD values span for Group 1 dataset was larger than Group 2, which was probably caused by the comparatively fewer sequenc- es (23 isolates in Group 1). CSFV Group 2 evolved much faster than Group 1, which indicated that Group 2 would be predominant in whole CSFV isolates and acquire supe-

RESULTS AND DISCUSSION

The respective evolutionary rate of Group 1 and Group 2 are shown in Table 3, and mean nucleotide substitution rate with 95% HPD are displayed in Fig 2. Our previous study reported that both CSFV Group 1 and Group 2 were both contributed to the epidemic of CSF in mainland China (2). However, it is noteworthy that Group 2 of CSFV’s, with a evolutionary rate of 3.6861×10⁻³ subs/site/
During the evolutionary process of CSFV, many factors would affect viral phylogeny including the immunological status of animals, the presence of wild reservoirs, inefficient vaccination campaigns as well as socio-economic factors (17). When the host immune defense change the viral population had to compete with the immune system adapting to keep track with the viral changes (18). Pigs have been vaccinated with the attenuated lapinized vaccine in China since mid-1950s. The vaccine strains of CSFV was classified into group 1 of highly virulent fatal strains and Group 2 consisting of moderately virulent isolates were responsible for the rising incidence of subacute and chronic CSF outbreaks (19). On the basis of the evidences, we presume that resistance against the host immune pressure would cause Group 2 to optimize its evolutionary strategy. An increased evolutionary rate under the constant selection pressure would be beneficial for the virus to escape the host immune response (20).

In brief, our work may be helpful to better understand the elevated evolutionary rates of CSFVs, however, the real mechanism behind the diversity in the evolutionary rate needs further investigation.

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REFERENCES

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<th>Group</th>
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<th>Mean substitution rate</th>
<th>95% HPD (highest probability density)</th>
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<tr>
<td>1</td>
<td>23</td>
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<td>3.1189\times10^{-5}-1.0118\times10^{-3}</td>
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<tr>
<td>2</td>
<td>45</td>
<td>3.6861\times10^{-3}</td>
<td>2.0816\times10^{-3}-5.5134\times10^{-3}</td>
</tr>
</tbody>
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Fig 2. Mean substitution rate with 95% HPD (highest probability density) in Group 1 (left) and Group 2 (right).

Table 3. Rates of CSFV nucleotide substitution in Group 1 and Group 2