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 (N^{pro}-C-E^{rns}-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B) (4). The polyprotein gives rise to autoprotease (N^{pro}), four structural proteins (C, E^{rns}, 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-

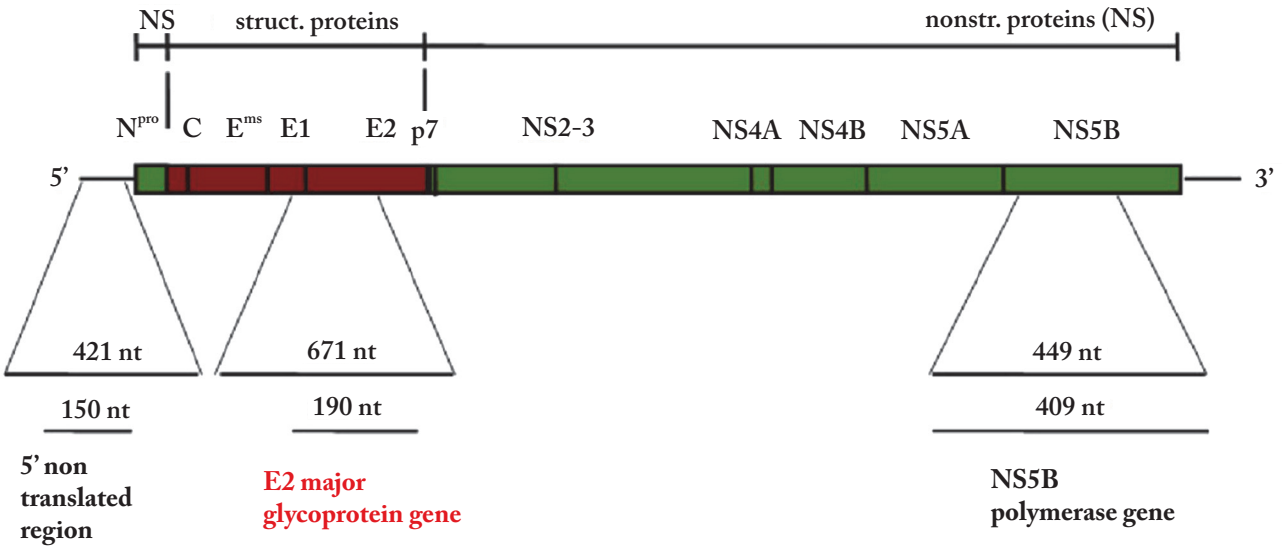

Fig 1. The CSFV genome structure and three variable regions (E2, NS5B and 5'NTR) could be used for evolutionary analysis. (Tajen from Paton *et al*., 2000, Vet. Microbiol. 137-157).

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 CSFV 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).

Table 1. The CSFVs Group 1 and Group 2

Accession No.	Years	Subgroup	Accession No.	Years	Subgroup
EF421652	1982	1.1	EF421690	2000	2.1
EF421651	1998	1.1	EF421691	1999	2.1
EF421639	1996	1.1	EF421692	2005	2.1
EF421649	2001	1.1	EF421693	1997	2.1
DQ127910	2004	1.1	EF421667	2000	2.1
EF421653	2006	1.1	EF421669	2001	2.1
EF421654	2006	1.1	AF143088	1998	2.1
EF421665	1998	1.1	EF421676	2002	2.1
EF421664	1999	1.1	EF421678	1998	2.1
EF421646	2002	1.1	EF421681	1999	2.1
EF421648	1995	1.1	EF421694	1998	2.1
EF421663	1998	1.1	EF421695	1999	2.1
EF421660	2002	1.1	EF683635	2005	2.1
EF421661	2002	1.1	EF683620	2007	2.1
EF421659	1998	1.1	EF421696	1998	2.1
EF421658	1998	1.1	EF421698	1999	2.1
FJ157213	2006	1.1	EF421699	2000	2.1
EF421656	2006	1.1	EF369431	2004	2.1
EF421979	1998	1.1	EF369444	2004	2.1
EF421645	2006	1.1	EF369429	2005	2.1
EF421644	1999	1.1	EF369439	2006	2.1
EF421642	1995	1.1	FJ157210	2006	2.1
EF421666	1999	1.1	FJ157211	2006	2.1
EF421700	1999	2.1	EF421682	1996	2.1
FJ456870	2004	2.1	EF421685	1999	2.1
FJ456871	2005	2.1	EF014334	2002	2.2
FJ456867	2006	2.1	EF421708	1995	2.2
FJ456868	2007	2.1	EF421709	1997	2.2
EF683627	2007	2.1	AF143082	1997	2.2
FJ607780	2008	2.1	AF143083	1998	2.2
FJ977628	2009	2.1	EF421710	1984	2.2
EF421655	2002	2.1	EF421980	1998	2.2
EF421688	2002	2.1	EF421707	1999	2.2
EF683612	2007	2.1	EF421983	1986	2.3

Sixty eight CSFV isolates (23 Group 1 isolates and 45 Group isolates) from the mainland China were used for evolutionary rate analysis.

Table 2. The best-fitting substitution model of CSFV Group 1 and 2

Group	Model	-lnL	AIC	Delta	Weight	cumWeight
1	**HKY**	383.9895	863.9791	5.9245	0.0122	0.9609
	HKY+I	383.4146	864.8293	6.7747	0.0080	0.9768
	HKY+G	383.4476	864.8952	6.8407	0.0077	0.9846
	HKY+I+G	383.451	866.902	8.8474	0.0028	0.9969
	GTR	380.4132	864.8263	6.7718	0.0080	0.9689
	GTR+I	379.8645	865.729	7.6744	0.0051	0.9896
	GTR+G	380.0103	866.0207	7.9661	0.0044	0.994
	GTR+I+G	379.5507	867.1013	9.0468	0.0026	0.9994
2	HKY	1068.6414	2321.2827	59.0629	0.0	1.0
	HKY+I	1055.319	2296.638	34.4182	0.0	1.0
	HKY+G	1055.7944	2297.5889	35.3691	0.0	1.0
	HKY+I+G	1055.946	2299.8921	37.6722	0.0	1.0
	GTR	1052.2368	2296.4736	34.2538	0.0	1.0
	GTR+I	1039.948	2273.896	11.6762	0.0020	1.0
	GTR+G	1036.9003	2267.8006	5.5808	0.0429	0.9566
	GTR+I+G	1037.6774	2271.3549	9.1351	0.0072	0.998

The less AIC value reflects the more fitting substitution model. Hasegawa-Kishino-Yano (HKY) model and the General Time Reversible with Gamma (GTR+G) model were determined as the best-fitting substitution model of CSFV of Group 1 and 2 (highlighted with bold), respectively. -lnL: negative log likelihood; AIC: akaike information criterion; delta: AIC difference; weight: AIC weight; cumWeight: cumulative AIC weight.

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/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 sequences (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 superiority in the evolutionary process against the host immune pressure and environmental selection pressure. This was in accordance with our previous report that Group 2 evolved for a much farther distance than Group 1. The higher substitution rates in Group 2 may imply the trend of switch from Group 1 to 2, which was directly supported by our phylogenetic analysis and previous reports (2), and could further explain why Group 2 viruses more extensively in many provinces of China.

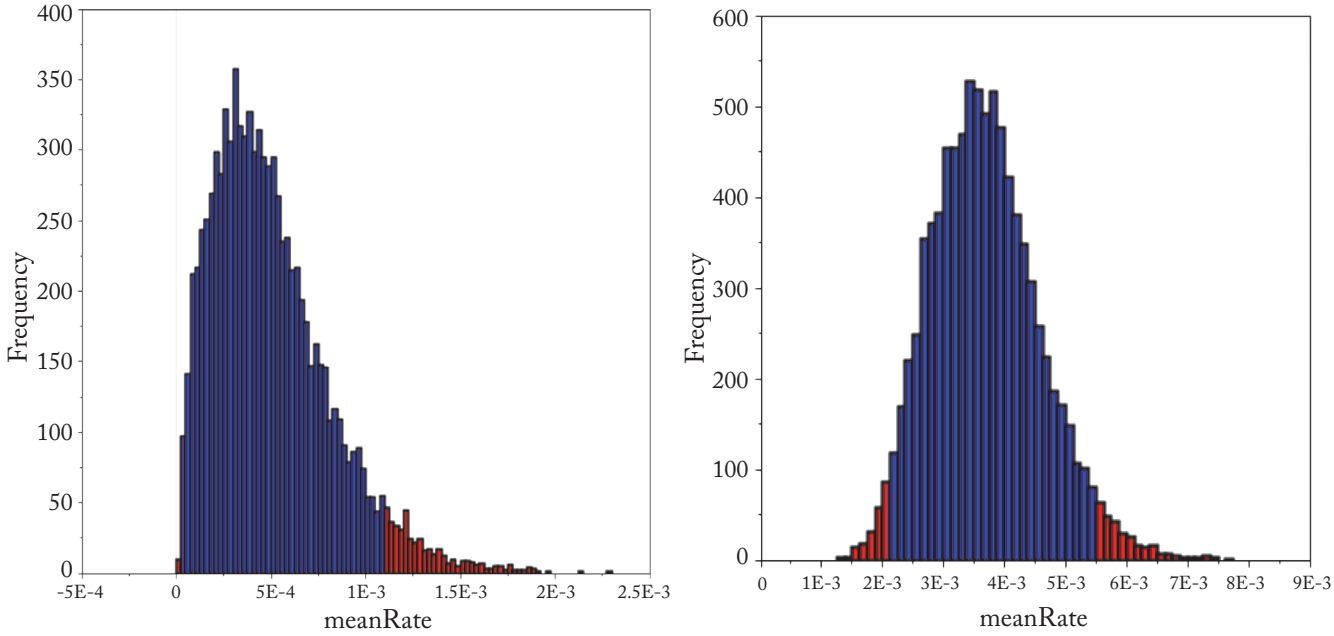

Fig 2. Mean substitution rate with 95% HPD (highest probability density) in Group 1 (left) and Group 2 (right).

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

Group	Nucleotide substation rate		
	CSFV sequences	Mean substitution rate	95% HPD (highest probability density)
1	23	4.9852×10^{-4}	3.1189×10^{-5}-1.1018×10^{-3}
2	45	3.6861×10^{-3}	2.0816×10^{-3}-5.5134×10^{-3}

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 outhe breaks (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 is 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

1. Camargos, M.F., Pereda, A., Stancek, D., Rocha, M.A., dos Reis, J.K.P., Greiser-Wilke, I. and Leite, R.C.: Molecular characterization of the env gene from Brazilian field isolates of Bovine Leukemia Virus. Virus Genes 34: 343-350, 2007.
2. Zhang, H., Wang, Y.H., Cao, H.W. and Cui, Y.D.: Phylogenetic analysis of E2 genes of classical swine fever virus in China. Isr J Vet Med 65: 151-155, 2010.
3. Fan, Y.F., Zhao, Q., Zhao, Y., Wang, Q., Ning, Y.B. and Zhang, Z.Q.: Complete genome sequence of attenuated low-temperature Thiverval strain of classical swine fever virus. Virus Genes 36: 531-538, 2008.
4. Singh, V.K., Saikumar, G., Bandyopadhyay, S.K. and Paliwal, O.P.: Phylogenetic analysis of classical swine fever virus (CSFV) by cloning and sequencing of partial 5 ' non-translated genomic region. Indian Journal of Animal Sciences 74: 1093-1097, 2004.
5. Blacksell, S.D., Khounsy, S., Boyle, D.B., Greiser-Wilke, I., Gleeson, L.J., Westbury, H.A. and Mackenzie, J.S.: Phylogenetic anal-

ysis of the E2 gene of classical swine fever viruses from Lao PDR. Virus Research 104: 87-92, 2004.
6. Tu, C.C., Lu, Z.J., Li, H.W., Yu, X.L., Liu, X.T., Li, Y.H., Zhang, H.Y. and Yin, Z.: Phylogenetic comparison of classical swine fever virus in China. Virus. Res. 81: 29-37, 2001.
7. Paton, D.J., McGoldrick, A., Greiser-Wilke, I., Parchariyanon, S., Song, J.Y., Liou, P.P., Stadejek, T., Lowings, J.P., Bjorklund, H. and Belak, S.: Genetic typing of classical swine fever virus. Vet. Micr. 73: 137-157, 2000.
8. Montesino, R., Toledo, J.R., Sanchez, B., Zamora, Y., Barrera, M., Royle, L., Rudd, P.M., Dwek, R.A., Harvey, D.J. and Cremata, J.A.: N-Glycosylation Pattern of E2 Glycoprotein from Classical Swine Fever Virus. Journal of Proteome Research 8: 546-555, 2009.
9. Qi, Y., Liu, L.C., Zhang, B.Q., Shen, Z., Wang, J. and Chen, Y.H.: Characterization of antibody responses against a neutralizing epitope on the glycoprotein E2 of classical swine fever virus. Archives of Virology 153: 1593-1598, 2008.
10. Chen, N., Hu, H.X., Zhang, Z.F., Shuai, J.B., Jiang, L.L. and Fang, W.H.: Genetic diversity of the envelope glycoprotein E2 of classical swine fever virus: Recent isolates branched away from historical and vaccine strains. Vet. Micr. 127: 286-299, 2008.
11. Liu, L.H., Xia, H.Y., Belak, S. and Widen, F.: Development of a primer-probe energy transfer real-time PCR assay for improved detection of classical swine fever virus. Journal of Virological Methods 160: 69-73, 2009.
12. Huang, Y.L., Pang, V.F., Pan, C.H., Chen, T.H., Jong, M.H., Huang, T.S. and Jeng, C.R.: Development of a reverse transcription multiplex real-time PCR for the detection and genotyping of classical swine fever virus. Journal of Virological Methods 160: 111-118, 2009.
13. Paton, D.J. and Greiser-Wilke, I.: Classical swine fever - an update. Research in Veterinary Science 75: 169-178, 2003.
14. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G.: The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-82, 1997.
15. Posada, D. and Crandall, K.A.: Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818, 1998.
16. Drummond, A.J. and Rambaut, A.: BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7: 214, 2007.
17. Duffy, S., Shackelton, L.A. and Holmes, E.C.: Rates of evolutionary change in viruses: patterns and determinants. Nature Reviews Genetics 9: 267-276, 2008.
18. Moennig, V., Floegel-Niesmann, G. and Greiser-Wilke, I.: Clinical signs and epidemiology of classical swine fever: A review of new knowledge. Vet. J. 165: 11-20, 2003.
19. He, C.Q., Ding, N.Z., Chen, J.G. and Li, Y.L.: Evidence of natural recombination in classical swine fever virus. Virus Research 126: 179-185, 2007.
20. Wonnemann, H., Floegel-Niesmann, G., Moennig, V. and Greiser-Wilke, I.: Genetic typing of classical swine fever virus isolates from Germany. Dtsch. Tierarztl. Wochenschr. 108: 252-256, 2001.