PHYLOGENETIC ANALYSIS OF E2 GENES OF CLASSICAL SWINE FEVER VIRUS IN CHINA

Zhang, H.,* Wang, Y.H., Cao, H.W. and Cui, Y.D
College of Biological Science and Technology, HeiLongJiang BaYi Agricultural University, DaQing 163319, China.

*Corresponding author:
Zhang Hua,
Tel: (086) 0459-6819290; Fax: (086) 0459-6819290;
E-mail: huazi8541@sina.com

ABSTRACT

In order to investigate the epidemiology of classical swine fever virus (CSFV) in China, the E2 glycoprotein gene of 68 CSFV's (domestic pigs) was isolated from mainland China during 1982-2009 and aligned, and a phylogenetic tree was constructed by the neighbor-joining (NJ) method using Phylip software package (version 3.68). Phylogenetic analysis of 190 nucleotides (nt) (located in 2518-2707) from E2 gene showed that 23 isolates were classified into Group 1 (subgroup 1.1) and 45 isolates were divided into Group 2, with 36 isolates being clustered into subgroup 2.1, subgroup 2.2 (8 isolates) and subgroup 2.3 (1 isolate). The results indicated that both CSFV Group 1 and Group 2 contributed to the epizootic of classical swine fever (CSF) in mainland China. Subgroup 2.1 virus population was more predominant in the economically developed southeast China along the coast whereas subgroup 1.1 mainly caused epizootics in inner provincial areas. Epidemiologic regional specificity might be caused by immune selection pressure between the two groups of CSFV.

Key words: classical swine fever virus, envelope glycoprotein E2, phylogenetic analysis

INTRODUCTION

Classical swine fever (CSF), a highly contagious disease of pigs and wild boars, causes significant economic losses in various parts of the world. The disease is characterized by fever and hemorrhage and can run either an acute lethal or a chronic course (1). Considering the economic importance of this disease, there is a need to investigate the epidemiology of classical swine fever virus (CSFV). One group of CSFV reflects a narrow range of evolutionary divergence, therefore genetic types of CSFV virus have been used to study CSFV epidemiology.

CSFV, bovine viral diarrhea virus (BVDV) type I and type II, and border disease virus (BDV) belong to the *Pestivirus* genus within the *Flaviviridae* family (2). The causative agent, CSFV is an enveloped virus with a single-stranded RNA genome of about 12.3kb in full length, which consists of 5′- and 3′- untranslated regions (UTR) flanking a single large open reading frame (ORF) coding for a polyprotein of about 3898 amino acids. The polyprotein is processed to four structural proteins (C, E^{rns}, E1, E2) and eight non-structural (N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins (3). As one of the major envelope glycoproteins, E2 protein always exists on the surface of viral particles in the form of E2 homodimers and E1-E2 heterodimers, whose molecular weight is 51-55kD and is composed of 373 amino acid residues. E2 protein is the most immunogenic among the CSFV proteins and not only induces neutralizing antibodies with high titers, but also plays a decisive role in cell tropism and virulence of CSFV (4).

As one of most variable region, N-terminal of E2 protein includes four domains A, B, C, D, with domain A being classified into three subdomains A1, A2, A3. In this variable region, the 190 nt of E2 gene located in 2518-2707 is extensively used for the genotyping and genetic comparison of CSFV's (5). Previous studies on the phylogenetic relationship of CSFV's have been divided into three main groups, and showed that all the isolates in mainland China belonged to Group 1 and 2 (6, 7). Except for 190 nt of E2 gene, the other two regions, NS5B and 5′ UTR, have been used for phylogenetic analysis and resulted in the same resolution (5, 8).

Although vaccination is extensively practiced in China during the last decades, there has been increased incidence of subacute and chronic diseases with a long duration, atypical clinical signs and relatively low morbidity outbreaks of disease (6). Meanwhile, it is reported that the population of the virus has showen the trend to switch from Group 1 to Group 2 in some countries of Europe and Asia (9, 10). It is well known that the Group 1 viruses are present in all the modified live vaccines and many of highly virulent strains which could cause acute CSF, while the Group 2 viruses are characterized by moderately virulent strains which cause the subacute and chronic CSF (5, 7). Thus a good understanding the evolutionary characteristics of the glycoprotein E2 of CSFV could provide clues for the switching of virus population in endemic areas. In current study, based on the analysis of the 190 nt E2 sequence, 68 CSFV's isolates from 21 provinces in China were investigated for phylogenetic relationship.

MATERIALS AND METHODS

Virus isolates

68 virus isolates were obtained form GeneBank website (http://www.ncbi.nlm.nih.gov/) and EU reference laboratory for CSFV database in Hannover. (http://viro08.tiho-hannover.de/eg/csf/startCSF.cgi) as shown in Table 1.

Phylogenetic analyses

The phylogenetic tree was calculated as described previously (3, 5). Briefly, 68 Chinese isolates (collected between 1982 to 2008), whose 190 nt sequences encompassing nucleotides 2518-2707, were aligned with 7 reference

strain sequences representing three groups as well as seven subgroups using Clustal X (version 1.83) (11). The software package Tree-Puzzle (version 4.0.2) was employed to estimate the value of transition/transversion ratio (12). Phylogenetic tree was constructed by the neighbor-joining (NJ) method with the modules Seqboot, Dnadist, Neighbor and Consense with Phylip software package (version 3.68) (13). For visualization and printing of the trees, the Treeview program (version 1.6.6) was applied (14). Strain Kanagawa served as outgroup, and the bootstrap values were estimated for 100 replicates (9).

RESULTS AND DISCUSSION

Despite intensive study, the understanding of the genetic diversity of different genotypes of CSFV remains uncertain. It is reported that the E2 gene of CSFV is under positive selection and the codon usage varies among different virus groups (15). In order to define the evolutionary relationships among CSFV's and genotypes of epidemic CSFV's in China, we applied the phylogenetic tree of E2 sequences (190 nt). Sixty eight Chinese isolates were segregated into two groups with 23 isolates being clustered into subgroup 1.1, 36 isolates in subgroup 2.1, 8 isolates in subgroup 2.2, and only 1 isolate, GX1/86, in subgroup 2.3 as displayed in Fig 1. The topology of CSFV's obtained in the present study was similar to that seen in several previously published phylogenies of the 190 nt of E2 region (6).

In subgroup 1.1, 23 Chinese CSFV isolates were divided clearly into two distinct genotypes (1.1a and 1.1b). Genotype 1.1a comprised 6 isolates with a main distribution in mid-southern region of China. More importantly, the genotype 1.1a were first isolated in 1982 (HeN1/82) and the following outbreaks occurred after 1995. In contrast subgroup 1.1b. has appeared in recent epizootics, and has appeared over a period of more than 10 years since the middle of 1990's comprising 17 isolates with a more extensive distribution. Furthermore, subgroup 1.1b was shown to be more closely related to the Italian isolates (Brescia, 1.1). This distribution of subgroup 1.1 aided our understanding of the evolution of subgroup 1.1 in China. Interestingly to date in China, none of the field viruses have tested into subgroup 1.2 and 1.3 within Group 1. Historical isolates of subgroup 1.2 only found in European counties (Czech, Poland, 1993) (5).

Unlike subgroup 1.1, subgroup 2.1 does not exhibit the further subdivision and has shown the widest geographical region in China. Several reports pointed out the same situation in the epidemic areas, where the historical group which has persisted for many years has become 'silent' and being replaced recently by Group 2 in Europe, Korea, and Taiwan (5, 9, 16, 17). Our molecular analysis correlated precisely with the epidemiology indicating that the 2.1 virus caused initial outbreaks in 1994 and then continuously caused infections since 1996. In contrast to the subgroup 2.1 responsible for the CSFV worldwide outbreaks thereafter, contemporaneous prevalence of subgroup 1.1 might be taken into consideration, where the 'silent' types resulted to some extent in a switch from Group 1 to Group 2. Nevertheless except for GS1/97 and GS2/98 in subgroup 2.2, the other 6 isolates resulted in outbreaks in the inshore province and frontier province. As the smallest subgroup, subgroup 2.3 was strictly distributed in south of China, especially in the southeast coastal province. To our surprise, subgroup 2.3 was identified as the one of most important CSFV isolates prevailing in many European countries in the past (18, 19), which was the clue for the international transmission of CSFV and indicated the possible epidemic trend in the future rooted in the economic trade of pig populations introduced form overseas. In addition, it is evident from tracing the epidemiology, genotype 3 CSFV has been absent from China and the genotype only appeared in the regions of Asia from southern Japan to Thailand (4, 6, 20), which did not result in any transmission of CSFV from Southeast Asia to China.

Previous studies revealed that Group 2, especially subgroup 2.1 was the main genotype that contributed to the epidemic of CSFV's during the last decades in China (4, 6). However, our data showed that 23 isolates were classified into subgroup 1.1. The results not only complemented the existing database about the virus isolates but also suggested that CSFV subgroup 1.1, recognized as a historical group, was still playing a vital role in the epidemic of CSFV in some interior areas in China. In brief, the contemporary existence of CSFV Group 1 and Group 2 with 4 subgroups was the epidemiological pattern in China. It was a noteworthy feature that most of subgroup 2.1 virus population was more predominant in economically developed southeast of china along the coast, whereas, subgroup 1.1 mainly caused epizootics in inner provincial regions. We presumed that that the immune selection pressure may be responsible for the regional specificity.

REFERENCES

1. Moennig V., Floegel-Niesmann G., and Greiser-Wilke I.: Clinical signs and epidemiology of classical swine fever: A review of new knowledge. Veterinary Journal 165: 11-20, 2003.
2. Paredes J.C.M., Oliveira E.A.S., Oliveira L.G., Rosa J.C.A., and Roehe P.M.: Serum neutralization as a differential serological test for classical swine fever virus and other pestivirus infections. Arquivo Brasileiro De Medicina Veterinaria E Zootecnia 51: 403-08, 1999.
3. Vilcek S., Stadejek T., Ballagi-Pordany A., Lowings J.P., Paton D.J., and Belak S.: Genetic variability of classical swine fever virus. Virus Res 43: 137-47, 1996.
4. 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. Veterinary Microbiology 127: 286-99, 2008.
5. 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. Veterinary Microbiology 73: 137-57, 2000.
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 Research 81: 29-37, 2001.
7. Li X.M., Xu Z.F., He Y.N., Yao Q.X., Zhang K.S., Jin M.L., Chen H.C., and Qian P.: Genome comparison of a novel classical swine fever virus isolated in China in 2004 with other CSFV strains. Virus Genes 33: 133-42, 2006.
8. Harasawa R., and Giangaspero M.: Genetic variation in the 5 ' end and NS5B regions of classical swine fever virus genome among Japanese isolates. Microbiology and Immunology 43: 373-79, 1999.

9. Pan C.H., Jong M.H., Huang T.S., Liu H.F., Lin S.Y., and Lai S.S.: Phylogenetic analysis of classical swine fever virus in Taiwan. Archives of Virology 150: 1101-19, 2005.
10. Wonnemann H., Floegel-Niesmann G., Moennig V., and Greiser-Wilke I.: Genetic typing of classical swine fever virus isolates from Germany. Deutsche Tierarztliche Wochenschrift 108: 252-56, 2001.
11. Meyers G., Rumenapf T., and Thiel H.J.: Molecular cloning and nucleotide sequence of the genome of classical swine fever virus. Virology 171: 555-67, 1989.
12. Strimmer K., and von Haeseler A.: Likelihood-mapping, a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci USA 94: 6815-19, 1997.
13. Felsenstein J.: Phylip, phylogeny inference package (version 3.5c). Cladistics 5: 164-66, 1989.
14. Page R.D.M.: TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357-58, 1996.
15. Tang F.Q., Pan Z.S., and Zhang C.Y.: The selection pressure analysis of classical swine fever virus envelope protein genes E-rns and E2. Virus Research 131: 132-35, 2008.
16. Sung J.H., Kang M.L., Kang S.G., Cha S.B., Lee W.J., Shin M.K., Roh Y.M., Kim B.H., and Yoo H.S.: Cloning, Sequencing and Phylogenetic Analysis of the Classical Swine Fever Virus E2 Glycoprotein Korean Isolates. 15th Congress of the Federation of Asian Veterinary Associations, Fava-Oie Joint Symposium on Emerging Diseases, Proceedings: P223-P24 112, 2008.
17. Deng M.C., Huang C.C., Huang T.S., Chang C.Y., Lin Y.J., Chien M.S., and Jong M.H.: Phylogenetic analysis of classical swine fever virus isolated from Taiwan. Veterinary Microbiology 106: 187-93, 2005.
18. Biagetti M., Greiser-Wilke I., and Rutili D.: Molecular epidemiology of classical swine fever in Italy. Veterinary Microbiology 83: 205-15, 2001.
19. Bjorklund H., Lowings P., Stadejek T., Vilcek S., Greiser-Wilke I., Paton D., and Belak S.: Phylogenetic comparison and molecular epidemiology of classical swine fever virus. Virus Genes 19: 189-95, 1999.
20. Parchariyanon S., Inui K., Pinyochon W., Damrongwatanapokin S., and Takahashi E.: Genetic grouping of classical swine fever virus by restriction fragment length polymorphism of the E2 gene. Journal of Virological Methods 87: 145-49, 2000.

TABLE

Table 1: The CSFV's isolates from the mainland China during the period 1982-2009

Provinces	Isolates	Accession No.	Years	Genotype	Provinces	Isolates	Accession No.	Years	Genotype
Beijing	BJ1/96	EF421639	1996	1.1	Hunan	HuN1/02	EF421655	2002	2.1
	BJ2/00	EF421667	2000	2.1		HuN2/02	EF421688	2002	2.1
	BJ3/01	EF421669	2001	2.1	Jiangsu	JS1/07	EF683612	2007	2.1
Guangdong	GD1/95	EF421642	1995	1.1	Jiangxi	JX1/06	EF421656	2006	1.1
Gansu	GS1/97	AF143082	1997	2.2	Jilin	JL1/97	EF421709	1997	2.2
	GS2/98	AF143088	1998	2.1		JL2/00	EF421690	2000	2.1
	GS3/98	AF143083	1998	2.2		JL3/06	FJ157213	2006	1.1
	GS4/99	EF421644	1999	1.1	Liaoning	LN1/84	EF421710	1984	2.2
	GS5/02	EF421676	2002	2.1	Ningxia	NX1/98	EF421658	1998	1.1
	GS6/06	EF421645	2006	1.1		NX2/98	EF421659	1998	1.1
Guangxi	GX1/86	EF421983	1986	2.3		NX3/99	EF421691	1999	2.1
	GX2/98	EF421979	1998	1.1	Qinghai	QH1/02	EF421660	2002	1.1
	GX3/98	EF421678	1998	2.1		QH2/02	EF421661	2002	1.1
	GX4/98	EF421980	1998	2.2		QH3/05	EF421692	2005	2.1
	GX5/99	EF421681	1999	2.1	Sichuan	SC1/97	EF421693	1997	2.1
	GX6/99	EF421707	1999	2.2		SC2/98	EF421663	1998	1.1
	GX7/02	EF014334	2002	2.2		SC3/98	EF421694	1998	2.1
	GX8/04	EF369431	2004	2.1		SC4/99	EF421664	1999	1.1
	GX9/04	EF369444	2004	2.1		SC5/99	EF421695	1999	2.1
	GX10/05	EF369429	2005	2.1	Shanghai	SH1/05	EF683635	2005	2.1
	GX11/06	EF369439	2006	2.1		SH2/07	EF683620	2007	2.1
Heilong Jiang	HLJ1/06	FJ157210	2006	2.1	Shanxi	SX1/98	EF421665	1998	1.1
	HLJ2/06	FJ157211	2006	2.1		SX2/98	EF421696	1998	2.1
Hainan	HaiN1/02	EF421646	2002	1.1		SX3/99	EF421698	1999	2.1
Hebei	HeB1/95	EF421648	1995	1.1		SX4/00	EF421699	2000	2.1
	HeB2/95	EF421708	1995	2.2	Yunnan	YN1/99	EF421666	1999	1.1
Henan	HeN1/82	EF421652	1982	1.1		YN3/99	EF421700	1999	2.1
	HeN2/96	EF421682	1996	2.1	Zhejiang	ZJ1/04	FJ456870	2004	2.1
	HeN3/98	EF421651	1998	1.1		ZJ2/05	FJ456871	2005	2.1
	HeN4/99	EF421685	1999	2.1		ZJ3/06	FJ456867	2006	2.1
	HeN5/01	EF421649	2001	1.1		ZJ4/07	FJ456868	2007	2.1
	HeN6/04	DQ127910	2004	1.1		ZJ5/07	EF683627	2007	2.1
Hubei	HuB1/06	EF421653	2006	1.1		ZJ6/08	FJ607780	2008	2.1
	HuB2/06	EF421654	2006	1.1		ZJ7/09	FJ977628	2009	2.1

68 CSFV isolates from the mainland China were used for construction of phylogenetic tree and evolutionary rate analysis. Virus code: capital letters represented the geographical provinces in China; i.e. BJ, Beijing; GD, Guangdong; GS, Gansu; GX, Guangxi; HLJ, Heilongjiang; HaiN, Hainan; HeB, Hebei; HeN, Henan; HuB, Hubei; HuN, Hunan; JS, Jiangsu; JX, Jiangxi; JL, Jilin; LN, Liaoning; NX, Ningxia; QH, Qinghai; SC, Sichuan; SH, Shanghai; SX, Shanxi; YN, Yunnan; ZJ, Zhejiang. The same capital letters followed by numbers indicated the isolates form one province and the last double-digital number indicated the years of isolation. Virus isolates were named in sequence next to the geographical province.

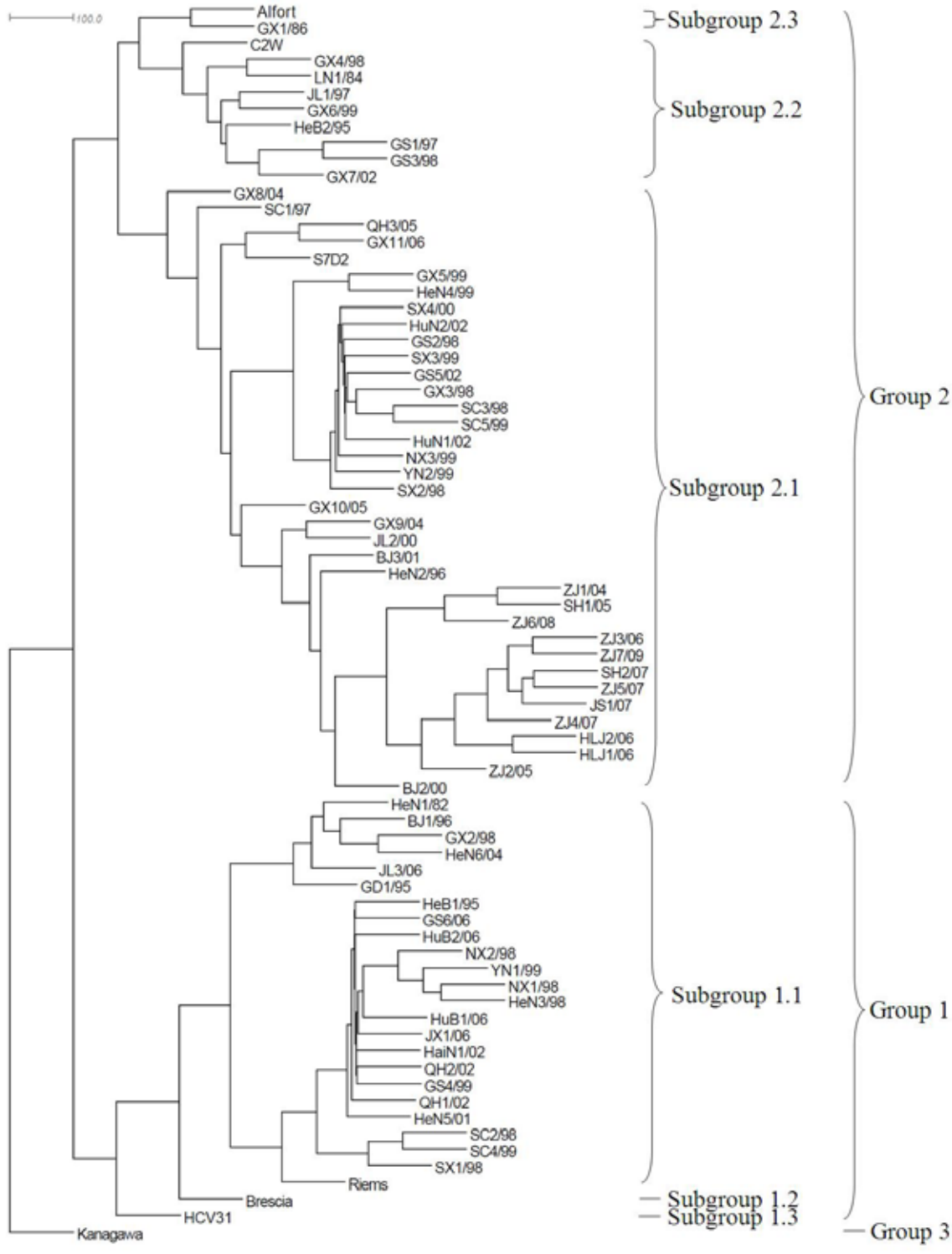

Fig.1. Neighbor-joining method of phylip software package (version 3.68) (13) was used for construction of phylogenetic tree on the basis of E2 gene variable region (190 nt). 68 Chinese CSFV isolates form 21 provinces and 7 reference strains (Riems/Germany, U45277, subgroup 1.1; Brescia/Italy, M31768, subgroup 1.2; HCV31/Honduras, AJ781098, subgroup 1.3; S7D2/Italy, L36171, subgroup 2.1; C2W/Italy, M36165, subgroup 2.2; Alfort/Germany, J04358, subgroup 2.3; Kanagawa/Japan, subgroup 3.4) retrieved from Genbank website and EU reference laboratory for CSFV database were analyzed. The value of transition/transversion ratio was estimated to be 6.38 with Tree-puzzle software (version 4.0.2) (12). Bootstrap values were estimated for 100 replicates, and Kanagawa strain was served as outgroup. Phylogenetic tree was visualized by Treview software (version 1.6.6) (14), and subgroups were shown in the right part of the corresponding grouping.