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Research paper

Genetic diversity of subgenotype 2.1 isolates of classical swine fever virus

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ABSTRACT

As the causative agent of classical swine fever, the economically devastating swine disease worldwide, classical swine fever virus (CSFV) is currently classified into the 11 subgenotypes, of which subgenotype 2.1 is distributed worldwide and showing more genetic diversity than other subgenotypes. Prior to this report, subgenotype 2.1 was divided into three sub-subgenotypes (2.1a–2.1c). To further analyze the genetic diversity of CSFV isolates in China, 39 CSFV isolates collected between 2004 and 2012 in two Chinese provinces Guangxi and Guangdong were sequenced and subjected to phylogenetic analysis together with reference sequences retrieved from GenBank. Phylogenetic analyses based on the 190-nt and/or 1119-nt full length E2 gene fragments showed that current CSFV subgenotype 2.1 virus isolates in the world could be divided into 10 sub-subgenotypes (2.1a–2.1j) and the 39 isolates collected in this study were grouped into 7 of them (2.1a–2.1c and 2.1g–2.1j). Among the 10 sub-subgenotypes, 2.1d–2.1j were newly identified. Sub-subgenotype 2.1d isolates were circulated only in India, however the rest 9 sub-subgenotypes were from China with some of them closely related to isolates from European and neighboring Asian countries. According to the temporal and spatial distribution of CSFV subgenotype 2.1 isolates, the newly classified 10 sub-subgenotypes were further categorized into three groups: dominant sub-subgenotype, minor sub-subgenotype and silent sub-subgenotype, and each sub-subgenotype can be found only in certain geographical areas. Taken together, this study reveals the complex genetic diversity of CSFV subgenotype 2.1 and improves our understanding about the epidemiological trends of CSFV subgenotype 2.1 in the world, particularly in China.

1. Introduction

Classical swine fever (CSF) is an extremely contagious swine disease with high morbidity and mortality, featuring symptoms of hemorrhagic fever and immunosuppression (Moennig et al., 2003). As an OIE notifiable disease, CSF caused significant economic losses in the pig industry worldwide. CSF virus is a member of the genus *Pestivirus* within the family *Flaviviridae*, which is an enveloped virus harboring a single strand positive-sense RNA genome (King et al., 2011). The genome is about 12.3 kb in size and consists of a large open reading frame encoding a polyprotein of 3898 amino acids, as well as the untranslated regions at both ends (5′UTR and 3′UTR). The polyprotein can be processed into 4 structural proteins (Core, E^{rns}, E1 and E2) and 8 non-structural proteins (N^{pro}, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) by viral and host proteases.

Phylogenetic analysis was extensively used for tracing CSF viruses and analyzing the epidemiological situation (Paton et al., 2000; Tu et al., 2001; Sabogal et al., 2006; Postel et al., 2012). CSFV isolates are currently divided into 3 genotypes, 11 subgenotypes (1.1–1.4; 2.1–2.3; 3.1–3.4) by phylogenetic analysis based on the nucleotide sequences of 5′UTR (150 nt), E2 (190 and 1119 nt) and NS5B (409 nt) (Paton et al., 2000; Postel et al., 2013). Among these subgenotypes, genotype 3 viruses previously circulating in Asia and the United Kingdom have no longer been reported in the last 10 years, while genotype 1 viruses mainly circulate in Latin American and Asia, but disappeared in Europe since the 1970s (Paton et al., 2000; de Arce et al., 2005; Greiser-Wilke et al., 2000; Jemeršić et al., 2003; Leifer et al., 2010; Postel et al., 2012, 2013; Sabogal et al., 2006). However, genotype 2 viruses are predominant worldwide with subgenotype 2.1 viruses mainly circulating in China and other Asian and European countries including Korea,

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Israel and Lithuania. Subgenotypes 2.2 and 2.3 previously present in China are still predominant in domestic pigs and wild boar in European countries, and the latter was further divided into sub-subgenotypes 2.3.1 and 2.3.2 (Blome et al., 2010). Since subgenotype 2.1 viruses were prevalent worldwide and caused significant economic losses for pig industries, more and more viruses of subgenotype 2.1 were sequenced and reported on GenBank, but detailed evolutionary analysis of these viruses has not been performed. Thus, it's important to systemically analyze the phylogeny of subgenotype 2.1 viruses.

Subgenotype 2.1 was first reported in China in 1979 and then in Malaysia in 1986 (Wang et al., 2000; Paton et al., 2000). The large CSF outbreaks in Europe in the late 1990s were also caused by subgenotype 2.1 viruses, which were later classified into subgenotype 2.1a (Greiser-Wilke et al., 2000; Jemeršić et al., 2003; Postel et al., 2012; Deng et al., 2005). Further analysis showed that the dominant type of CSF viruses in Taiwan changed from subgenotype 3.4 to subgenotype 2.1 in the late 1990s and the latter was first segregated into sub-subgenotypes 2.1a and 2.1b (Deng et al., 2005). In addition, the dominant CSF virus in Korea shifted from subgenotype 3.2 to 2.1b (Cha et al., 2007). Chen et al. reported that 34 out of 35 CSFV isolates collected from Southeast China between 2004 and 2007 were classified into sub-subgenotype 2.1b (Chen et al., 2008; Sun et al., 2013). In addition, subgenotype 2.1 isolates (2.1a and 2.1c) were also found to be distributed in Lao DR (Blacksell et al., 2004). More recently, 2.1c isolates has emerged from Hunan and Guangdong provinces since 2011 (Jiang et al., 2013a, 2013b). CSF viruses collected in Lithuania between 2009 and 2011 were grouped into 2.1 as well as those from Israel in 2009, these viruses were closely related to Chinese isolates (Postel et al., 2012; David et al., 2011), but the involved sub-subgenotype (s) were not described. Recently, isolates from wild boars in India since 2011 were also subgenotype 2.1 (Rajkhowa et al., 2014; Ahuja et al., 2015), but their sub-subgenotype classification was not analyzed. Taken together, subgenotype 2.1 viruses showed significant genetic diversity and wide sub-subgenotypes may exist in addition to 2.1a–2.1c.

To improve our understanding of the genetic diversity and distribution of subgenotype 2.1 viruses, phylogenetic characterization based on the 190-nt and full-length E2 gene of 39 CSFV isolates from Guangdong and Guangxi provinces, China, together with reference viruses, was conducted, and 10 sub-subgenotypes (2.1a–2.1j) have been identified in present study. Except subgenotype 2.1d is from India, the isolates from China are grouped into other 9 sub-subgenotypes of subgenotype 2.1 and each subgenotype is found in certain geographic regions.

Table 1
Thirty-nine CSF viruses isolated from Guangxi and Guangdong provinces.

Isolate name	Year of isolation	Location of isolate	Genotype	GenBank accession no.
GXNN-2/04[a]	2004	Guangxi	2.1a	EF369431
GXTD-1/05[a]	2005	Guangxi	2.1a	EF369432
GXBB-3/06[a]	2006	Guangxi	2.1a	EF369443
GXNN-1/04[a]	2004	Guangxi	2.1i	EF369444
GXNN-5/06[a]	2006	Guangxi	2.1i	EF369433
GXNN-6/06[a]	2006	Guangxi	2.1i	EF369436
GXCZ-1/11[a]	2011	Guangxi	2.1i	JQ713127
GXLA-1/12[a]	2012	Guangxi	2.1i	KC502864
GXFS-1/05[a]	2005	Guangxi	2.1c	EF369428
GXFC-1/06[a]	2006	Guangxi	2.1g	EF369439
GXFC-2/06[a]	2006	Guangxi	2.1g	EF369441
GXFC-3/06[a]	2006	Guangxi	2.1g	EF369434
GXFC-4/06[a]	2006	Guangxi	2.1g	EF369430
GXGL-1/06[a]	2006	Guangxi	2.1g	EF369442
GXBB-1/05[a]	2005	Guangxi	2.1h	EF369429
GXNN-7/06[a]	2006	Guangxi	2.1h	EF369437
GXPN-1/06[a]	2006	Guangxi	2.1h	EF369438
GXBB-5/06[a]	2006	Guangxi	2.1h	EF369440
GXXY-2/06[a]	2006	Guangxi	2.1h	EF369435
GXHX-1/11[a]	2011	Guangxi	2.1b	KC502865
GXNN-1/12[a]	2012	Guangxi	2.1b	KC502865
GD176/2011[b]	2011	Guangdong	2.1b	KT853107
GD45/2011[b]	2011	Guangdong	2.1b	KT853111
GD349/2011[a]	2011	Guangdong	2.1j	KT853119
GD41/2011[b]	2011	Guangdong	2.1i	KT853116
GD317/2011[b]	2011	Guangdong	2.1i	KT853109
GD318/2011[b]	2011	Guangdong	2.1i	KT853110
GD148/2011[b]	2011	Guangdong	2.1i	KT853105
GD156/2011[b]	2011	Guangdong	2.1i	KT853106
GD18/2011[b]	2011	Guangdong	2.1g	KT853114
GD19/2011[b]	2011	Guangdong	2.1g	KT853112
GD12/2011[b]	2011	Guangdong	2.1g	KT853113
GD143/2011[b]	2011	Guangdong	2.1g	KT853115
GD24/2011[a]	2011	Guangdong	2.1c	KT853117
GD68/2011[a]	2011	Guangdong	2.1c	KT853118
GD191/2011[b]	2011	Guangdong	2.1c	KT853108
GD26/2011[b]	2011	Guangdong	2.1c	KT853102
GD52/2011[b]	2011	Guangdong	2.1c	KT853103
GD53/2011[b]	2011	Guangdong	2.1c	KT853104

[a] The partial E2 gene (190 nt) of CSF viruses was sequenced and analyzed;
[b] The full-length E2 gene (1119 nt) of CSF viruses was sequenced and analyzed.

2. Materials and methods

2.1. Viruses and sequences

Thirty-nine kidney samples of classical swine fever-suspected pigs were collected between 2004 and 2012 from 39 farms in Guangdong and Guangxi provinces, China. These clinical samples were from sick pigs showing hemorrhage, high fever and typical pathological lesions in lymph nodes, spleen, kidney, and lung (Table 1). E2 sequences of other CSFV genotypes used for phylogenetic analysis in the present study were retrieved from GenBank, which were reported from China, Korea, Mongolia, India, Lao PDR, Germany, Croatia, Russia, Indonesia, Israel and United Kingdom.

2.2. Amplification and sequencing of E2 gene

For PCR amplification of 190-nt and/or 1119-nt E2 gene, total RNA of the infected kidney tissue was extracted using Trizol reagent (Ambion, Carlsbad, CA) according to the manufacturer's instruction and subsequently reverse transcribed with Super Script III first strand cDNA synthesis kit (Invitrogen, Carlsbad, CA) using random primers. The obtained cDNA was served as template to amplify 1119 nt full-length E2 (2440 nt–3558 nt) or 190 nt (2517 nt–2706 nt) E2 gene fragment. For amplification of full-length E2 gene (1119 nt), forward primer CSFV-E2-F: 5′-GGYRAATATGTGTGTGTWAGACC-3′ and reverse primer: CSFV-E2-R: 5′-TGGTCTTRACTGGRTTGTTRGTC-3′ were used for PCR, the position of the amplified fragment is from 2210 to 3706 nt referred to the complete genome sequence of Alfort187 strain. Amplification of 190-nt E2 was performed using PCR with 671-nt E1/E2 fragment as the template, and the same primers were used in previous report (Paton et al., 2000). PCR amplification was performed using AccuPrime Taq DNA Polymerase High Fidelity according to the manufacturer's instruction (Invitrogen, Carlsbad, CA). The PCR products containing 190 nt or 1119 nt were analyzed by 1% agarose gel electrophoresis and the positive amplicon was then sequenced commercially. To obtain the consensus sequence, at least three independent sequencing for each amplicon were performed.

2.3. Phylogenetic analysis

Alignment of nucleotide/amino acid sequences of 190-nt or 1119-nt E2 gene were conducted using CLC sequence viewer 7.6.1 (QIAGEN, Germany). Phylogenetic analysis were performed using Molecular Evolutionary Genetics Analysis software MEGA 6.06 (Center for Evolutionary Functional Genomics, Tempe, AZ). Neighbor-joining method including Bootstrap value of 1000 repetitions was used for construction of phylogenetic tree.