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Phylogenetic analysis of classical swine fever virus isolated from Taiwan

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Abstract

By analyzing the E2 sequences of classical swine fever virus from field outbreaks in Taiwan during 1993–2001, three virus populations with distinct genotypes were determined including one historical (subgroup 3.4) and two exotic (subgroup 2.1) strains. The first subgroup 2.1 virus was isolated in 1994 and further sporadic outbreaks occurred after 1996. Phylogenetic analysis using the E2 region has segregated the Taiwanese strains of 2.1virus into two different genotypes (termed 2.1a and 2.1b). The 2.1b viruses were only isolated in 2001 and shared approximately 94.8% nucleotide identities to the 2.1a viruses in the total genomic sequences. The results suggest that the 2.1a and 2.1b viruses may be introduced from different origins.

Keywords: Classical swine fever virus; *Pestivirus*; Epidemiology; Phylogenetics

1. Introduction

Classical swine fever (CSF) is the most insidious and devastating disease of swine and wild boars, causing significant economical losses in the pig industry over most regions of the world. CSF is caused by classical swine fever virus (CSFV), a member of the genus *Pestivirus* within the family Flaviviridae. The genome of CSFV comprises a single open reading frame (ORF), approximately 12.3 kb in length (Meyers et al., 1989; Meyers and Thiel, 1996). This ORF, flanked by a 5′-untranslated region (UTR) and a 3′-UTR, encodes a polyprotein composed of about 3898 amino acids, which is processed by viral and cellular enzymes into four structural (C, E0, E1 and E2) and eight nonstructural (N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins (Thiel et al., 1991; Meyers and Thiel, 1996).

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CSF viruses consist of one serotype, reflecting a narrow range of evolutionary divergence (Vanderhallen et al., 1999). Therefore, genetic typing of the virus has been used in understanding the evolution, spread of viruses and the origins of disease outbreaks. Three regions, 5′-UTR, E2 and NS5B, in the viral genome are most extensively used for genetic analysis and for studying virus diversity on the basis of sequence homology (Hofmann et al., 1994; Lowings et al., 1996; Stadejek et al., 1996; Greiser-Wilke et al., 1998; Paton et al., 2000). Analysis using a 96 nt region of the 5′-UTR, a 190 nt region of the E2 and a 409 nt region of the NS5B has resulted in similar resolution to classify CSFV into three major groups and their subgroups. Group 1 and its three subgroups (1.1, 1.2 and 1.3) comprise most of the historical strains (Lowings et al., 1996; Paton et al., 2000) distributed in most regions of the world. Group 2 containing most of the current viruses, which segregates into subgroup 2.1, 2.2 and 2.3, has increased activity and caused epidemic infection since the 1980s (Paton et al., 2000). The earliest 2.1 strain (VRI2277) was isolated from Malaysia in 1986 (Vilcek et al., 1996; Paton et al., 2000). In the 1990s, the 2.1 viruses have caused epidemic of outbreaks in Germany (Oleksiewicz et al., 2003), The Netherlands (Widjojoatmodjo et al., 1999; Stegeman et al., 2000), Switzerland, Austria, Italy, Belgium, Spain (Paton et al., 2000), China (Tu et al., 2001) and Taiwan. Nevertheless, Group 3 contains disparate viruses distributed in regions such as Taiwan, Korea, Japan, Thailand and the United Kingdom (Sakoda et al., 1999; Paton et al., 2000).

Although an attenuated lapinized live vaccine (LPC) has been used to protect pigs from CSF since the 1950s in Taiwan, outbreaks occur endemically. We have genetically analyzed the viruses obtained during 1993–2001. Our results not only identify that the 3.4 strains are the historical strains in Taiwan, which may become silent strains in fields after 1996, but also reveal that there has been a switch in the virus populations from the 3.4 to the 2.1 strains circulating wild types.

2. Materials and methods

2.1. Virus isolates

A total of 36 isolates of CSFV recovered from field outbreaks in domestic pig herds over a period of 9 years (1993–2001) in Taiwan (Table 1) were included in this study. These isolates were passaged twice in PK-15 cells and identified with fluorescence-labelled specific antiserum against CSFV. Virus information on their geographical origins (prefecture), years of isolation and genotypes was summarized in Table 1. Two of the 36 isolates, p97/FL/94 and 94.4/IL/94, have previously been studied by other investigators, and the relevant references are cited where applicable (Shiu et al., 1996; Paton et al., 2000). In addition, 59 strains of E2 nucleotide sequences retrieved from published data were listed in Fig. 1.

2.2. RNA amplification and sequencing

Nucleotide sequences in the E2 and NS5B regions were amplified by RT-PCR from cultured virus suspension. Viral RNA was extracted from 140 μl of cultured suspension using QIAamp Viral RNA Mini Kit (QIAGEN) by the method recommended by the manufacturers. For E2 amplification, viral RNA was reverse-transcribed using the antisense primer (5′-TGTCTCATTTGCCAAGATGCACTT-3′, position 3134–3111) and then amplified for 30 cycles (denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s) using the sense primer (5′-TGAGGGATTTRACYAGRGTCTGGA-3′, position 2317–2337). For NS5B amplification, the sense primer (5′-TGACCATGCACATGTCAGAAGTACC-3′, position 11,053–11,077) and the antisense primer (5′-TATCCTTCTAATCAGTGGGTTCCAG-3′, position 11,576–11,600) were used. The amplified product was purified using the QIAquick PCR Purification Kit (QIAGEN). Subsequently, DNA fragments were sequenced by the direct sequencing method, using primers as in the PCR amplification and using BigDye™ Terminator Cycle Sequencing Kit. The samples were loaded on an Applied Biosystems 3100 sequencer (Foster City, CA).

2.3. Phylogenetic analysis

Phylogenetic analysis was initially carried out on the *E2* gene a 190 nt region encompassing nucleotides 2518–2707 (Lowings et al., 1996; Paton et al., 2000) and the *NS5B* gene, a 409 nt region spanning nucleotides 11,158–11,566 (Bjorklund et al., 1999;