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Research Article

Molecular Characterization of the Porcine Epidemic Diarrhea Virus TW4/2014 in Taiwan

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Abstract

Infections by Porcine Epidemic Diarrhea Virus (PEDV) have been shown to be significantly correlated with fatal diarrhea in suckling piglets. Although PEDV was first identified in Europe, since late 2010, it has become increasingly problematic on several continents, including Asia, North America, and South America. Since late 2013, several outbreaks of PEDV have emerged in Taiwan. Analysis of the partial PEDV S gene sequences in these strains revealed that these outbreaks were in the same clade as US strains of PEDV. To elucidate the molecular characterization of the Taiwanese and reference strains in greater detail, the full length of a local isolate (TW4/2014) that was derived from a suckling piglet was sequenced using next-generation sequencing techniques and compared to other worldwide strains. The complete genome size of TW4 (27,966 nucleotides excluding the 5' leader sequence) appears to be almost identical (99.9%) to several of the 2013 US strains. Our results indicate that the recent PEDV isolates from Taiwan share a common evolutionary origin with US-like PEDV strain from several countries (South Korea, Canada, Mexico, and Peru) and US strains.

Keywords: PEDV; Porcine epidemic diarrhea; Complete genome; Next-generation sequencing

Abbreviation

PEDV: Porcine Epidemic Diarrhea Virus; HCoV: Human Coronavirus; TGEV: Transmissible Gastroenteritis Virus; ORFs: Open Reading Frames; nts: Nucleotides; PCV2: Porcine Circovirus Type 2.

Introduction

Porcine Epidemic Diarrhea Virus (PEDV) is an enveloped virus with a large, capped and polyadenylated RNA genome of approximately 28,000 nucleotides [1]. PEDV belongs the genus *Alphacoronavirus*, family *Coronaviridae*, order *Nidovirales*. Other members of this subgroup include Human Coronavirus (HCoV) 229E, HCoV NL63, and bats coronavirus 512/05 [2]. Although PEDV was first identified in Europe, since 2010, it has become increasingly problematic on several continents, including Asia, North America, and South America [1,3-5].

Starting in April 2013, PEDV was first identified in the United States. All of the affected swine farms experienced explosive epidemics of diarrhea and vomiting affecting pigs of all ages, with 90-95% mortality in suckling piglets [4]. Since that time, US strain-like PEDV variants have become prevalent in several countries, including South Korea [5,6], Canada [7], Mexico [8], and Peru [9]. Whole-genome sequencing of the 2013 US PEDV revealed the highest identity with the Chinese strain AH2012 [10].

Since late 2013, several outbreaks of PEDV infection have emerged in Taiwan. Suckling piglets under 2 weeks of age showed severe vomiting and watery yellowish diarrhea with high morbidity and mortality. Analysis of the partial PEDV S gene sequence revealed that these outbreaks were in the same clade as the US strains of PEDV [3]. However, the full length of a Taiwanese PEDV strain remains to be analyzed.

Materials and Methods

Animal

PEDV TW4 was isolated in January 2014 from a one-day-old piglet with naturally occurring PED. Within 24 hours of birth, this piglet developed watery yellow diarrhea, weight loss and dehydration. Thinned and distended small intestine walls with watery yellowish contents were recorded during necropsy. The clinical specimens were negative for rotavirus and Transmissible Gastroenteritis Virus (TGEV) and positive for the partial PEDV S gene using reverse transcription polymerase chain reaction [3].

Isolation of viral RNA and sequencing for complete genome analysis

Total nucleic acid was extracted from the piglet's intestine using the MagNA Pure LC 2.0 (Roche Diagnostics, Mannheim, Germany) following the manufacturer's protocol. The nucleic acid templates were then sequenced for the whole genome using the MiSeq sequencing system (Illumina Inc, San Diego, CA, USA).

Sequence analysis

The complete sequences of PEDV TW4 were then compared with reference strains, and the results are summarized in Table 1. Multiple alignments of nucleic acid sequences were performed using the Clustal W methods within the MegAlign program (DNASTAR

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Table 1: Sequence identity of PEDV TW4 and reference strains.

	Isolation			
Virus	Year	Country	Sequence identity	GenBank accession no.
PEDV CV777	1978	Belgium	96.9	NC003436
PEDV DR13	1999	South Korea	97.7	JQ023161
PEDV LZC	2006	China	96.6	EF185992
PEDV JS2008	2008	China	97.4	KC210146
PEDV SM98	2011	South Korea	96.7	GU937797
PEDV CHGD-01	2011	China	98.3	JX261936
PEDV CH/FJND-3	2011	China	99.0	JQ282909
PEDV AH2012	2012	China	99.6	KC210145
PEDV CH/YNKM-8	2013	China	99.0	KF761675
PEDV IA2	2013	USA	99.9	KF468754
PEDV Iowa/18984	2013	USA	99.9	KF804028
PEDV MN	2013	USA	99.9	KF468752
PEDV MEX/104	2013	Mexico	99.8	KJ645708
PEDV VN/KCHY-310113	2013	Vietnam	98.1	KJ960180
PEDV OH851	2014	USA	99.1	KJ399978
PEDV K14JB01	2014	South Korea	99.8	KJ623926
PEDV ON-018	2014	Canada	99.9	KM189367

Table 2: Coding potentials and gene size in PEDV TW4/2014.

ORFs	Start-end (nucleotide position)	No. of nucleotides	No. of amino acids
5' UTR	1-220	220	-
ORF 1a	221-12,529	12,309	4103
ORF 1ab	221-20,565 (shift at 12,610)	20,345	6781
S	20,562-24,722	4161	1387
ORF 3	24,722-25,396	675	225
E	25,377-25,607	231	77
M	25,615-26,295	681	227
N	26,307-27,632	1326	442
3' UTR	27,633-27,966	334	-

Inc., WI, USA). The phylogenetic analyses were conducted using the maximum likelihood method within MEGA 5, version 5.05.

Results

Genomic sequence of PEDV TW4

The full genomic RNA sequence of PEDV TW4 comprises 27,966 nucleotides (nts), excluding the 5' leader sequences. Sequence analysis revealed that PEDV TW4 contains several conserved open reading frames (ORFs) with an overall genome organization similar to known PEDV strains (Table 2). The overall nucleotide composition is as follows: A, 24.8%; C, 19.1%; G, 22.7%; and T, 33.4%. The G+C content is 41.8 %.

5', 3' Untranslated regions (UTRs) and ORFs

The 5' UTR of PEDV TW4 comprises 220 nts, identical to other known PEDV strains; the 3' UTR of our virus comprises 334 nts, which is also identical to other PEDV strains. The 5' two-thirds of the genome contain the 1a (nt 221-12,529) and 1ab (nt 221-20,565) genes that encode the nonstructural polyproteins. A typical coronavirus "slip site," 5'-UUUAAAC-3' (nt 12,610–12,616), is located within this gene. These genes are followed by genes encoding the four structural proteins: spike (nt 20,562-24,722), envelope (nt 25,377-25,607), membrane (nt 25,615-26,295), and nucleocapsid (26,307-27,632) (Table 2). The accessory gene (ORF3: nt 24,722-25,396) identified in all of the known PEDV strains was also found in PEDV TW4 (Table 2).

Genetic comparison and phylogenetic analysis with reference PEDV strains

The overall sequence comparison revealed that PEDV TW4 was more closely related to the known subgroup 1b CoV but not 1a (TGEV Purdue and Feline coronavirus NTU156) within the *alphacoronaviruses* (Figure 1). PEDV TW4 was not clustered with the prototypical PEDV CV777. In contrast to the low nucleotide sequence similarity between Chinese strain AH2012 (99.6%) and recent Taiwanese strains, the homology levels between this PEDV TW4 isolate and US strains (99.9%) appeared to be much higher (Table 1 and Figure 2).

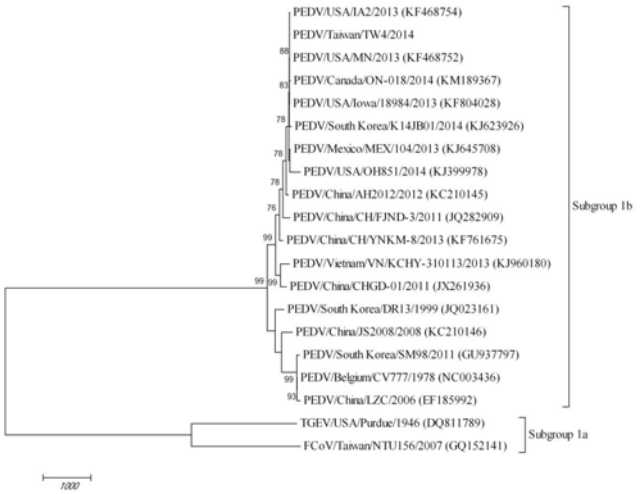

Figure 1: Phylogenetic relationships constructed using the complete genome sequences of PEDV TW4/2014 and reference strains. The analysis was performed using the maximum likelihood method based on 1,000 replicates within the MEGA 5 software. Bootstrap support values greater than 75 are shown. The complete genome sequence of transmissible gastroenteritis virus (TGEV) and feline coronavirus (FCoV) were included as an outgroup in this study.

Discussion

PEDV has recently become an economic concern in the swine industry in Asia, North America, and South America [1,3-6,8,9]. In Taiwan, several outbreaks of PEDV infection have emerged since late 2013. Suckling piglets under 2 weeks of age show severe vomiting and watery yellowish diarrhea with high morbidity and mortality. Our previous study suggested that this outbreak of viruses clustered in the same clade as the US strains according to the partial S gene analysis [3]. This is the first report of a complete PEDV genome in Taiwan. Interestingly, comparative genome analysis of reference PEDV isolates revealed that the complete genome sequences of recent Taiwanese strains were almost identical (99.9%) to several of the 2013 US strains (PEDV IA2, Iowa/18984, and MN) (Table 1).

A previous study suggested that the US PEDV strains were most closely related to a strain isolated in 2012 in China (AH2012) [10]. Although the complete genome sequence of PEDV TW4 clustered with the US strains and AH2012, TW4 was more closely related to the US strains with high bootstrap values. Such US-like PEDV isolates have not only been observed in Taiwan [3] but also in other countries, including South Korea [5,6], Canada [7], Mexico [8], and Peru [9]. Taken together, our results indicate that recent PEDV outbreaks share a common evolutionary origin with PEDV strains throughout most of the swine industry. Those US-like PEDVs seen like highly virulent in piglets in several countries. Specific treatments of PEDV are not available [1]. Therefore, strict biosecurity measures should be established.

These US-like strains of the virus might have gained entry into different countries via unknown routes as early as late 2013. Spray-Dried Porcine Plasma (SDPP) has been suspected and tested for the presence of PEDV genome by real-time PCR [7,11]. Although feed that tested positive for the PEDV genome did not result in obvious piglet infection, contaminated feed still cannot be ruled out as a source of PEDV introduction in the field [7].

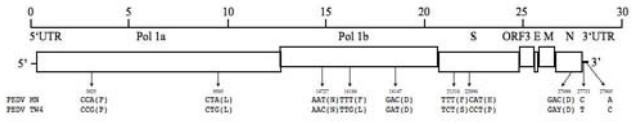

Figure 2: Schematic comparison of the complete genome between PEDV MN and PEDV TW/4. The arrows indicate the different features on the sequence. Brackets enclose the amino acids. Numerical position on the genome of PEDV MN, accession number KF468752.

These US-like PEDV variants are highly virulent in piglets in Taiwan. Several pig farms are still facing re-emergences of these US-like PEDV strains despite several cycles of feedback with pooled homogenized intestines from suckling pigs (data not shown). The reasons for this feedback failure need to be further investigated, including infection with Porcine Circovirus Type 2 (PCV2) in sows. The clinical course of PEDV disease is markedly affected by transplacental infection with PCV2 according to previous report [12]. In addition, the key variations in the amino acid sequences need to be further studied, including in the S proteins, which plays a crucial role in receptor binding and eliciting protective immunity [13]. Similar to most RNA viruses, coronaviruses mutate at a high frequency due to the high error rate during RNA polymerization. In addition, a unique feature of coronavirus genetics is the high frequency of RNA recombination in the natural evolution of this virus [14]. Therefore, additional PEDV cases need to be investigated using continuous surveillance and complete genome analysis to gain better evolutionary insight into PEDV.

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