NOTE *Virology*

US-Like Strain of Porcine Epidemic Diarrhea Virus Outbreaks in Taiwan, 2013–2014

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(Received 25 February 2014/Accepted 19 May 2014/Published online in J-STAGE 5 June 2014)

ABSTRACT: Since late 2013, several outbreaks of porcine epidemic diarrhea virus (PEDV) infection have emerged in Taiwan. Suckling piglets under 2 weeks of age showed severe vomiting and watery yellowish diarrhea with morbidity and mortality ranging from 80 to 100% and 90 to 100%, respectively. A total of 68 samples from 25 pig farms were confirmed as positive for PEDV and negative for rotavirus and transmissible gastroenteritis virus by reverse transcription PCR, and the partial S gene of PEDV was analyzed. Phylogenetic analysis places all 18 Taiwanese PEDV isolates collected during this outbreak in the same clade as the US strains of PEDV. This novel PEDV is prevailing and currently causing severe outbreaks in Taiwan.

KEY WORDS: diarrhea, disease outbreaks, porcine epidemic diarrhea virus

doi: 10.1292/jvms.14-0098; *J. Vet. Med. Sci.* 76(9): 1297–1299, 2014

Porcine epidemic diarrhea viruses (PEDVs) are enveloped viruses with a large, capped and polyadenylated RNA genome of approximately 28,000 nucleotides [6]. PEDVs belong to the genus *Alphacoronavirus*, family *Coronaviridae* and order *Nidovirales*. Other members of this subgroup include human coronavirus (HCoV) 229E, HCoV NL63 and Bats coronavirus 512/05 [8]. Although PEDV was first identified in Europe, it has become increasingly problematic in many Asian countries [6] and North America [7]. However, there is no retrospective study on the emergence of PEDV in Taiwan.

Pigs of all ages can be affected and can exhibit degrees of diarrhea and loss of appetite, which vary according to age. In suckling piglets that exhibit watery diarrhea, dehydration with milk curd vomitus and thin-walled intestines with severe villus atrophy and congestion, PEDVs are associated with disease. The disease can progress to death within a few days. The outbreak is distinguished by a nearly 100% prevalence of illness among piglets after birth and death rates of 80–100%. Boars and sows with mild diarrhea, vomiting and anorexia for a few days were reported to recover within a week [6, 7].

The spike (S) protein of coronavirus plays key roles in receptor binding for viral entry and neutralizing antibody induction for protective immunity [2]. CO-26K equivalent (COE) from the S protein of PEDV contains epitopes that are capable of inducing PEDV-neutralizing antibodies [1]. COE was found to be 1,495–1,914 bp of the coding sequence of spike protein gene of the PEDV strain Brl/87 [1].

Prior to late 2013, the prevalence of PEDV infection was relatively low with only sporadic infections. However, starting in December 2013, a remarkable increase in PEDV outbreaks occurred in Taiwan.

Samples from 32 pig farms in central and southern Taiwan from December 2013 through January 2014 were submitted to the animal hospital of National Pingtung University of Science and Technology. All of the pig farms had similar disease histories, clinical signs and lesions, including the presence of a sudden outbreak and rapid spread in farms, high mortality in young pigs and diseases affecting animals of all age, notably, vomiting and diarrhea. Piglets under 2 weeks of age showed severe vomiting and watery yellowish diarrhea with morbidity and mortality ranging from 80 to 100% and 90 to 100%, respectively. However, sows, gilt, finishing, growing and nursery pigs only developed appetite loss, anorexia and soft feces for 3–7 days with no mortality during this outbreak. The infected suckling pigs showed different degrees of weight loss and dehydration. Thinned and distended small intestine walls with watery yellowish contents were recorded during necropsy. Microscopically, marked enterocyte exfoliation and vacuolation consistent with villi atrophy and blunt were observed in the jejunum and ileum sections. Additionally, the clinical specimens were collected and tested for rotavirus [3], transmissible gastroenteritis virus [4] and porcine epidemic diarrhea virus [5]. All of the specimens were negative for rotavirus and transmissible gastroenteritis virus. A total of 68 samples from 25 pig farms were confirmed as positive for PEDV by reverse transcription PCR, and the partial S gene of PEDV was further analyzed [5]. The year of sampling, age and clinical history of each sampled pig are summarized in Table 1.

The COE domain of PEDV was amplified by RT-PCR as described by Kim *et al*. [5]. Nucleotide and deduced amino acid sequences of the COE domain of the 20 PEDV isolates and reference PEDV isolates were aligned and analyzed with

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Table 1. The clinical history of 20 porcine epidemic diarrhea virus isolates collected from Taiwanese pigs

Strain[a)]	Sampling time	County (Region)[b)]	Age[c)]	Specimen[d)]	Accession number
TW1/A	2013/OCT	Pingtung (S)	3 W	I	KJ434294
TW2/B	2013/DEC	Pingtung (S)	Sow	F	KJ434295
TW3/C	2013/DEC	Pingtung (S)	5 W	I	KJ434296
TW4/D	2014/JAN	Pingtung (S)	1 D	I	KJ434297
TW5/E	2014/JAN	Yunlin (C)	8 W	F	KJ434298
TW6/E	2014/JAN	Yunlin (C)	8 W	F	KJ434299
TW7/F	2014/JAN	Yunlin (C)	Sow	F	KJ434300
TW8/F	2014/JAN	Yunlin (C)	Boar	F	KJ434301
TW9/G	2014/JAN	Pingtung (S)	2 W	I	KJ434302
TW10/F	2014/JAN	Yunlin (C)	2 W	F	KJ434303
TW11/H	2014/JAN	Pingtung (S)	2 W	F	KJ434304
TW12/I	2014/JAN	Yunlin (C)	1 D	I	KJ434305
TW13/I	2014/JAN	Yunlin (C)	1 D	I	KJ434306
TW14/I	2014/JAN	Yunlin (C)	1 D	I	KJ434307
TW15/I	2014/JAN	Yunlin (C)	1 D	I	KJ434308
TW16/I	2014/JAN	Yunlin (C)	1 D	I	KJ434309
TW17/I	2014/JAN	Yunlin (C)	1 D	I	KJ434310
TW18/J	2014/JAN	Pingtung (S)	3 D	I	KJ434311
TW19/J	2014/JAN	Pingtung (S)	3 D	I	KJ434312
TW20/K	2014/JAN	Pingtung (S)	3 D	I	KJ434313

a) The letters in each name indicate the specific pig farm. b) Region of Taiwan. S: Southern Taiwan; C: Central Taiwan. c) Age of the pig at presentation. W: weeks; D: days. d) Specimen type. I: intestine; F: feces.

Clustal W using the MegAlign program (DNASTAR, Madison, WI, U.S.A.). The phylogenetic analyses were conducted with the maximum likelihood method using MEGA 5, version 5.05. All 18 PEDV isolates collected from this outbreak shared 99.5–100% DNA sequence identity of the partial S gene. Two major clusters, based on the phylogenetic relation of the partial nucleotide sequences of the COE domain in the S gene, were detected (Fig. 1). The first cluster was comprised of prototype isolates (TW1/A/2013 and TW2/B/2013) and Chinese strains LJB/03 and DX/2007. The second cluster consisted of all 18 Taiwanese isolates from this outbreak and 7 US isolates (Fig. 1). One nonsynonymous mutation was observed in recent Taiwanese PEDV strains and US strains at nucleotide positions 1,540–1,542 (GTA) of the S gene compared with PEDV CV777 (GTC), PEDV AH2012 (GTC) or the Taiwanese prototype strains TW1/A/2013 and TW2/B/2013 (GTC; Data not shown). Our findings demonstrate that the recent PEDV isolates in Taiwan were genetically similar to US PEDV strains rather than Chinese strains within the COE domain.

Our results indicate that the recent Taiwanese PEDV strains are closely related to those isolated from the U.S.A. These strains were responsible for the recent PEDV outbreak in Taiwan and produced a similar mortality rate and pathologic effects of US isolates. The US-like strain of the virus might have gained entry into Taiwan via unknown routes as early as December 2013. Taken together, our findings indicate that these PEDV outbreaks in Taiwan share a common evolutionary origin with the S gene of US strains of PEDV.

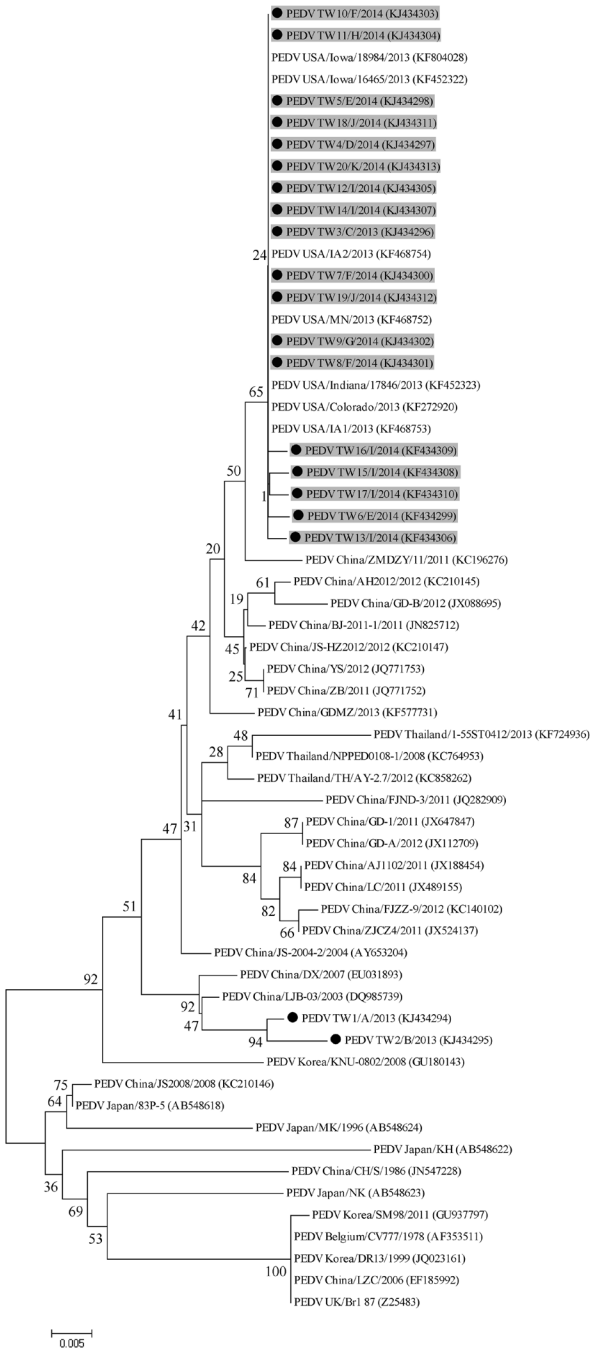

Fig. 1. Phylogenetic relationships based on the partial COE domains of the S genes of Taiwanese PEDV isolates and reference strains. The analysis was performed employing the maximum likelihood method based on 1,000 replicates using MEGA 5 software. Light grey underlay: Taiwanese PEDVs isolated in this severe outbreak. Solid circles: Taiwanese PEDV isolates in the present study.

The phylogenetic relationship and sequence analyses of the Taiwanese PEDV strains indicate that the recent Taiwan PEDV isolates differed from previous Taiwanese PEDV

isolates. Our data suggest that all recent Taiwanese PEDV isolates are genetically similar to US isolates identified in 2013. Additional PEDV cases should be investigated using continuous surveillance and sequence analysis.

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