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Genome Sequencing and Analysis of a Porcine Delta Coronavirus from Eastern China

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Abstract

Porcine delta coronavirus (PDCoV) has been reported in many countries, including the United States, Canada, South Korea, China, Thailand, Vietnam and Laos. In December 2016, clinical diarrhea similar to that caused by porcine epidemic diarrhea virus (PEDV), but with a lower mortality rate, was reported on a swine farm in Shanghai, China. 6 Intestine samples were collected from dead suckling piglets (<3 weeks old) with clinical diarrhea, and they were assayed for the presence of swine enteric coronaviruses. Polymerase chain reaction results were positive for PDCoV (6/6), but negative for PEDV (0/6), transmissible gastroenteritis virus (TGEV) (0/6) and porcine rotavirus group A (Rota A) (0/6). The full-length genome sequence of the PDCoV strain SHJS/SL/2016 was determined. Phylogenetic trees demonstrated that PDCoV strain SHJS/SL/2016 belongs to the Chinese clade, which might share a common evolutionary ancestor with United States and South Korean clades, but it clustered separately from Thai and Laotian PDCoV strains. This report describes the complete genome sequence of SHJS/SL/2016, and the data will promote a better understanding of the molecular epidemiology and genetic diversity of PDCoV isolates in China.

Keywords: Porcine deltacoronavirus; Full-length genome; Phylogenetics

Introduction

Porcine deltacoronavirus (PDCoV) is an enveloped, single-stranded, positive-sense RNA virus that is taxonomically classified within the family Coronaviridae, genus Deltacoronavirus [1]. The virus was first identified through a genomic sequence analysis of avian and pig isolates in Hong Kong in 2012 [2]. PDCoV was first detected in farmed pigs with diarrhea in the United States in early 2014 [3]. PDCoV causes an enteric disease that is characterized by watery diarrhea, dehydration, low mortality in adult pigs, and high mortality in piglets. The clinical symptoms of PDCoV disease are very similar to those of porcine epidemic diarrhea, but PDCoV disease is milder [4]. Since April 2017, disease caused by PDCoV strains has been reported in North America (the United States, Canada, and Mexico) [5-10] and Asia, including South Korea, Mainland China, Thailand, Laos, and Vietnam [11-17].

The PDCoV genome is approximately 25.4 kb in length (excluding the poly(A) tail), and starting from the 5′ end, approximately three-fourths of the viral genome encodes two overlapping open reading frames (ORFs) (ORF1a and ORF1b) that produce up to 15 nonstructural (NS) proteins, although PDCoV lacks the gene encoding NS protein 1 (NS1) [2]. Downstream of ORF1, PDCoV contains six additional ORFs (ORF2 to ORF7) that encode the spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins, as well as NS6 and NS7. The genome is flanked by short 5′ and 3′ untranslated regions (UTRs), with the typical gene order 5′-UTR-ORF1-S-E-M-NS6-N-NS7-3′-UTR [18].

To reveal the characteristics of this virus and determine more precisely its relationships with other PDCoV strains that have been reported in other countries, we determined and analyzed the complete genomic sequence of the SHJS/SL/2016 strain.

Methods

In December 2016, an outbreak of diarrhea occurred among piglets in a breeding farm in Shanghai. To determine etiology, 6 intestinal samples from dead suckling piglets (<3 weeks old) were collected. For the extraction of viral RNA from intestinal samples, suspensions were prepared by vortexing 1 g of intestines with 1 ml of phosphate-buffered saline (0.1M, pH 7.2). The suspensions were clarified at 5000× g for 10 minutes at 4°C. 200 µl of clarified supernatants was used to extract viral RNA by Viral RNA minikits (Qiagen, Germany) according to the manufacturer's instructions. Two pairs of primers (41F: 5′-TTTCAGGTGCTCAAAGCTCA-3′ and 735R: 5′-

GCGAAAAGCATTTCCTGAAC-3') were used for the detection of PDCoV nucleocapsid (N) gene with reaction conditions (50°C for 30 min and 95°C for 15 min for the reverse transcription reaction, followed by 40 cycles of PCR amplification at 95°C for 15 s, 55°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 7 min) [3]. In addition, molecular detection of the three diarrhea-related enteric viruses (Porcine epidemic diarrhea virus, PEDV; Porcine transmissible gastroenteritis virus, TGEV; Porcine rotavirus group A, Rota A) was performed by using the commercial real time RT-PCR kits (Weiboxin, Guangzhou, China) for further evaluation of the possible co-infection status with PDCoV in investigated pig samples. The complete genomic sequence of PDCoV (SHJS/SL/2016) was subsequently determined from extracted RNA by RT-PCR amplification of 16 regions covering the PDCoV genome as described previously [3]. At least three independent PCR amplicons were sequenced to obtain a consensus sequence. Sequences were assembled and analyzed using the DNASTAR software package (DNASTAR Inc., Madison, WI, USA). Phylogenetic trees were constructed by the neighbor-joining method using MEGA software version 5 [19]. The topology of the trees based on the nt sequences was obtained by majority-rule consensus using 1,000 bootstrap replicates, which are shown as percentages, and bootstrap values greater than 60% were considered statistically significant for grouping.

Results and Discussion

The PCR results demonstrated that all samples were positive for PDCoV (6/6), and none were PCR positive for PEDV (0/6), TGEV (0/6) or Rota A (0/6). After that, the full-length genome sequence of a PDCoV strain (SHJS/SL/2016) was determined. The newly characterized sequence has been deposited in the GenBank database under the accession number MF041982.

The complete genomic sequence of the SHJS/SL/2016 strain is 25,414 nt in length, excluding the poly(A) tail, and it consists of the 539-nt 5' UTR, the 18,797-nt replicase gene (nt 540 to 11,408 for 1a and nt 11,408 to 19,336 for 1b), the 3,480-nt S gene (nt 19,318 to 22,797), the 252-nt E gene (nt 22,791 to 23,042), the 654-nt M gene (nt 23,035 to 23,688), the 285-nt NS6 gene (nt 23,688 to 23,972), the 1,029-nt N gene (nt 23,993 to 25,021), the 603-nt NS7 gene (nt 24,087 to 24,689), and the 393-nt 3' UTR. A pairwise comparison showed that the complete genome shared 97.6%–99.3% nucleotide identities with the other 60 PDCoV strains available in GenBank. The highest level of similarity, shared with strain CHN-JS-2014, was 99.3 % (**Table 1**). A comparison of individual regions indicated that the S gene is the region of the viral genome that varies the most between strain SHJS/SL/2016 and the other PDCoV strains, with nucleotide sequence identities of 96.5%–99.5%, which are more variable than those of the NS7 gene (97.0%–99.0%) (**Table 1**). Moreover, a subsequent sequence alignment showed that seven PDCoV strains, including three Thai PDCoV strains, two Vietnamese PDCoV strains, one Laotian PDCoV strain, and one Chinese PDCoV strain, contain a 6-nt (TTTGAA) deletion and a 9-nt deletion (CCGGTTGGT) in ORF1a, while the SHJS/SL/2016 and CHN/Tianjin/2016 PDCoV strains only have a 6-nt deletion in ORF1a (**Figure 1**). Compared with these PDCoV strains, a 3-nt (TAA) deletion was observed in the S gene of PDCoV strain SHJS/SL/2016, and this deletion was also present in most Chinese PDCoV strains, except PDCoV strains HKU15-44 and CHN-AH-2004 (**Figure 1**). However, this strain has a 1-nt (T) insertion in its 3' UTR (**Figure 1**).

Table 1: Percent nucleotide sequence identity of SHJS/SL/2016 to the corresponding sequences of 60 PDCoV strains.

GenBank NO.	Virus	Complete genome (nt)	Complete Genome	5'UTR	ORF1a	ORF1b	S	E	M	NS6	N	NS7	3'UTR
KP757890	CHN-AH-2004	25420	98.9	99.8	98.6	99.4	98.4	98.8	98.8	99.6	98.6	95.7	99.2
JQ065042	HKU15-44	25430	98.9	99.8	98.4	99.2	99.1	99.6	98.6	99.3	98.9	98.3	99
JQ065043	HKU15-155	25425	99.1	99.1	98.8	99.5	99.1	99.6	98.9	99.6	99.3	99	99.5
KT266822	CH/Sichuan/S27/2012	25404	99.2	100	98.9	99.6	98.9	98.8	98.6	99.3	99.2	98.8	99
KT336560	CHN-HN-2014	25420	99	98.3	98.8	99.4	98.6	98.8	98.8	99.6	99	98.7	99.2
KP757892	CHN-JS-2014	25420	99.3	99.6	98.9	99.7	99.5	99.2	98.9	99.6	99.1	98.7	99.2
KP757891	CHN-HB-2014	25420	99.2	99.8	99	99.6	99.2	99.6	98.6	99.3	98.8	98.2	99
KU981059	NH	25420	99	99.6	98.7	99.5	99.1	99.2	98.9	98.9	98.8	98.5	98.7
KR131621	PDCoV/CHJXNI2/2015	25438	98.9	98.9	98.7	99.2	99.1	99.2	98.9	98.6	98.9	98.5	98.2
KT021234	CH/SXD1/2015	25419	98.9	99.3	98.8	99.4	98.5	99.2	98.9	98.9	98.6	97.8	98.2
KY065120	CHN/Tianjin/2016	25413	99	99.4	99.1	99	98.6	99.2	98.6	98.9	98.8	98.5	97.7
KM820765	KNU14-04	25422	98.9	99.6	98.6	99.4	99	99.2	98.3	99.6	98.8	98.7	98.7

KU051641	PDCoV/Swine/ Thailand/ S5011/2015	25405	97.6	99.1	97.4	98.1	96.5	99.6	98.2	98.2	97.3	97.5	96.7
KU051649	PDCoV/Swine/ Thailand/S5015L/ 2015	25405	97.6	99.3	97.4	98.1	96.5	99.6	98.2	98.2	97.3	97.5	96.7
KU984334	TT_1115	25403	97.6	99.1	97.4	98.1	96.6	99.6	97.9	98.2	98	97	96.7
KX834351	PDCoV/Swine/ Vietnam/ HaNoi6/2015	25406	97.9	99.4	97.6	98.3	96.8	99.2	98.8	98.6	98.5	97.8	98.2
KX834352	PDCoV/Swine/ Vietnam/ Binh21/2015	25406	97.9	99.4	97.7	98.3	96.9	99.2	98.8	98.6	98.5	97.8	98.2
KX118627	P1_16_BTL_0115 /PDCoV/2016/Lao	25405	97.6	99.1	97.4	98.1	96.7	99.6	98.2	98.2	98.2	97.3	96.7
KR265853	USA/Minnesota/ 2013	25394	99	99.6	98.6	99.4	98.9	99.2	98.5	99.6	99	98.8	99
KJ481931	PDCoV/USA/ Illinois121/2014	25406	98.9	99.4	98.6	99.4	99	99.2	98.5	99.6	98.7	98.5	99.2
KR265865	USA/ Iowa459/2014	25394	98.9	99.4	98.6	99.4	98.9	98.8	98.3	99.6	98.9	98.8	99.2
KJ601777	PDCoV/USA/ Illinois133/2014	25408	99	99.6	98.6	99.5	98.9	99.2	98.5	99.3	98.9	98.7	99
KJ601778	PDCoV/USA/ Illinois134/2014	25404	99	99.6	98.6	99.5	98.9	99.2	98.5	99.3	98.9	98.7	99
KJ601779	PDCoV/USA/ Illinois136/2014	25404	98.9	99.4	98.6	99.4	99	99.2	98.5	99.6	98.7	98.5	99.2
KJ601780	PDCoV/USA/ Ohio137/2014	25404	99	99.6	98.6	99.4	98.9	99.2	98.5	99.6	98.9	98.7	99.2
KJ462462	OH1987	25422	99	99.4	98.6	99.4	98.9	99.2	98.3	99.6	98.9	98.7	99.2
KR265861	USA/ Nebraska210/201 4	25404	99	99.4	98.6	99.5	98.9	99.2	98.3	99.3	98.8	98.7	99
KR265860	USA/ Nebraska209/201 4	25396	99	99.4	98.6	99.5	98.9	99.2	98.3	99.3	98.9	98.7	99
KR265859	USA/ Minnesota159/20 14	25401	99	99.6	98.6	99.5	99.1	99.2	98.3	99.6	99	98.8	99
KJ584355	IL2768	25422	99	99.6	98.6	99.4	99	99.2	98.5	99.3	99	98.8	99
KJ569769	IN2847	25422	99	99.4	98.6	99.4	98.9	99.2	98.5	99.3	99	98.8	99
KJ584358	PA3148	25422	99	99.4	98.6	99.4	99	99.2	98.2	99.6	99	98.8	99
KJ584356	SD3424	25422	98.9	99.6	98.6	99.4	98.9	99.2	98.3	99.6	98.7	98.5	98.7
KJ567050	8734/USA-IA/ 2014	25422	98.9	99.6	98.6	99.4	98.9	98.8	98.5	99.3	98.9	98.7	99
KJ584359	NE3579	25422	99	99.4	98.6	99.5	98.9	99.2	98.3	99.3	98.9	98.7	99
KR265857	USA/ Illinois273/2014	25394	98.9	99.6	98.6	99.4	99	98.8	98.3	99.6	98.9	98.7	99.2
KR265856	USA/ Illinois272/2014	25399	98.9	99.6	98.6	99.4	99	98.8	98.3	99.6	98.9	98.7	99.2
KJ769231	SdCV/USA/ OhioCVM1/2014	25422	98.9	99.6	98.6	99.4	98.9	99.6	98.3	99.6	98.5	98.2	99.2

KR265847	USA/ Minnesota442/2014	25394	98.9	99.4	98.6	99.5	98.9	99.2	98.2	99.6	98.7	98.5	98.7
KJ584357	KY4813	25422	99	99.6	98.6	99.5	99	99.2	98.5	99.6	99	98.8	98.7
KR265848	USA/ Minnesota214/2014	25396	98.9	99.6	98.6	99.5	98.9	99.2	98.2	99.6	98.7	98.5	98.7
KR265864	USA/ Minnesota292/2014	25395	98.9	99.4	98.6	99.4	99	99.2	98.3	99.6	98.9	98.8	99
KM012168	Michigan/ 8977/2014	25411	98.9	99.6	98.6	99.5	98.9	99.2	98.3	99.6	98.9	98.8	99
KJ620016	MI6148	25422	99	99.6	98.6	99.4	99	99.2	98.3	99.3	99	98.8	99.2
KR265862	USA/ Ohio444/2014	25394	98.9	99.4	98.6	99.4	98.9	99.2	98.3	99.6	98.8	98.7	99.2
KR265863	USA/ Ohio445/2014	25394	98.9	99.4	98.6	99.4	98.8	99.2	98.3	99.6	98.7	98.5	99.2
KR265850	USA/ Michigan448/2014	25394	99	99.6	98.6	99.5	98.9	99.2	98.5	99.6	99	98.8	99
KR265849	USA/ Michigan447/2014	25393	99	99.6	98.6	99.5	98.9	99.2	98.5	99.6	99	98.8	99
KR265852	USA/ Illinois449/2014	25394	99	99.6	98.6	99.4	99	99.2	98.5	99.3	99	98.8	99
KR265858	USA/ NorthCarolina452/2014	25394	98.9	99.6	98.5	99.4	98.9	99.2	98	99.6	99	98.8	99
KT381613	OH11846	25422	99	99.6	98.6	99.4	99	99.2	98.5	99.6	99	98.8	99.2
KR265851	USA/ Indiana453/2014	25394	99	99.6	98.6	99.5	99	99.2	98.2	99.6	98.9	98.7	99
KR265855	USA/ Minnesota455/2014	25394	98.9	99.6	98.6	99.4	98.8	99.2	98.5	99.6	98.9	98.7	99.2
KR265854	USA/ Minnesota454/2014	25394	99	99.6	98.6	99.5	98.9	99.2	98.5	99.6	98.9	98.7	99.2
KP981395	USA/IL/ 2014/026PDV_P11	25422	98.9	99.6	98.6	99.5	98.9	99.2	98.3	99.6	98.9	98.8	99
KR150443	USA/ Arkansas61/2015	25398	98.9	99.6	98.5	99.4	98.9	99.2	98.5	99.6	98.9	98.7	99.2
KX022602	PDCoV/USA/ Iowa136/2015	25382	98.9	99.4	98.5	99.4	98.8	99.2	98	99.6	98.9	98.7	98.7
KX022604	PDCoV/USA/ Nebraska137/2015	25382	98.9	99.4	98.5	99.4	98.9	99.2	98.2	99.6	98.8	98.7	98.9
KX022603	PDCoV/USA/ Minnesota140/2015	25394	98.9	99.4	98.5	99.4	98.9	99.2	98.2	99.6	98.9	98.7	98.7
KX022605	PDCoV/USA/ Nebraska145/2015	25320	98.9	99.4	98.5	99.4	98.8	99.2	98.2	99.6	98.9	98.7	98.1

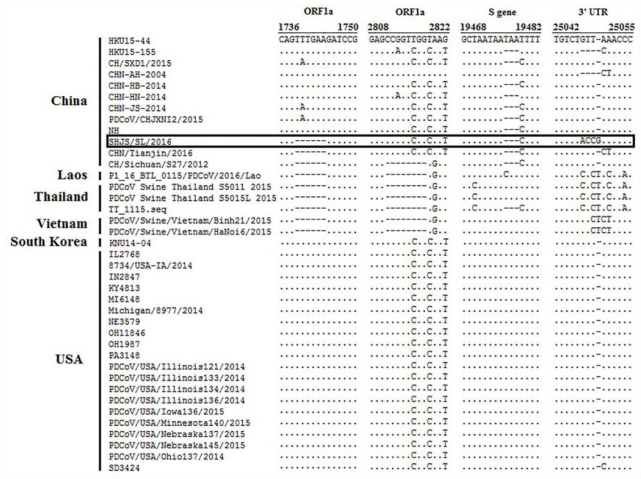

Figure 1: Four main deletions or insertions in the complete genome alignment. A multiple sequence alignment was constructed with ClustalW using DNASTAR software. PDCoV strain SHJS/SL/2016 is indicated in bold and highlighted with a box. A dot (•) indicates that the nucleotide exactly matches the consensus sequence. A dash (-) indicates that the nucleotide is deleted relative to the reference sequence.

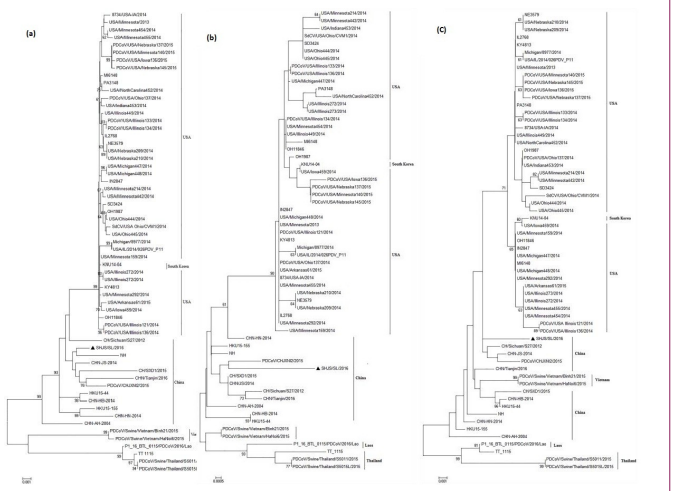

Figure 2: Phylogenetic analysis using the neighbor-joining method based on nucleotide sequences of different genes (A, S; B, M; C, N) of PDCoVs. Bootstrapping for 1,000 replicates with a value >60 % was performed to determine the percentage reliability of each internal node. The scale bar indicates the number of nucleotide substitutions per site. The sequence of the SHJS/SL/2016 strain is indicated by a black triangle.

A pairwise comparison of the nucleotide identities of 61 global PDCoV strains is summarized in **Table 1**. Moreover, the S, M and N genes were further analyzed. The S gene encodes a predicted protein of 1,159 amino acids. It contains 3,480-nt and, therefore, it is 3-nt shorter than that of the PDCoV reference strain HKU15-44. Based on the S gene, the SHJS/SL/2016 strain is closely related to Chinese PDCoVs, with 98.4%–99.5% and 99.1%–99.4% nucleotide and amino acid sequence identities, respectively (**Table 1**). It also shared sequence similarity with US PDCoV strains, with 98.8%–99.1% and 99.1%–99.3% identities at the nucleotide and amino acid levels, respectively (**Table 1**). The SHJS/SL/2016 PDCoV strain shared the lowest nucleotide homologies (96.5%–96.6%) with Thai PDCoV strains (**Table 1**). A phylogenetic analysis demonstrated that the PDCoV strains from the United States and South Korea clustered into a large clade, whereas PDCoV strain SHJS/SL/2016 clustered with other PDCoV strains detected in China since 2014, which suggests that the United States and South Korean clades might share a common evolutionary ancestor with the Chinese clade (**Figure 2A**). Interestingly, the PDCoV strains from Thailand, Laos, and Vietnam clustered in a distinct clade (**Figure 2A**). These findings are similar to those of previous studies [11,14,20].

The M gene is 654 nt long, and it encodes a protein of 217 amino acids. It has no nt deletions or insertions, but it does contain point mutations. The SHJS/SL/2016 PDCoV strain shared the highest nucleotide homologies (98.3%–98.9%) with the Chinese PDCoV strains, and the lowest nucleotide homologies (97.9%–98.2%) with the Thai PDCoV strains (**Table 1**). As shown in **Figure 2B**, the topology of the phylogenetic tree constructed using the M gene sequences of strain SHJS/SL/2016 and the other PDCoV strains was identical to that obtained with the S gene sequences.

The N gene is 1,029 nt in length, encoding a polypeptide of 342 amino acids. A nt sequence analysis revealed that there are no deletions or insertions in the N gene of any of the PDCoV strains. The nucleotide and amino acid sequences of the SHJS/SL/2016 PDCoV strain were 98.1%–98.7% and 98.5%–99.1% identical, respectively, to those of the Chinese PDCoV strains, and 97.8%–98.1% and 98.2%–99.1% identical, respectively, to those of the United States PDCoV strains (**Table 1**). The SHJS/SL/2016 PDCoV strain shared the lowest nucleotide homologies (97.3%–98.0%) with the Thai PDCoV strains (**Table 1**). As shown in **Figure 2C**, the phylogenetic tree constructed using the N gene sequences of SHJS/SL/2016 and the other PDCoV strains differed significantly from those obtained with the S and M genes. Chinese strains, including SHJS/SL/2016, formed a Chinese clade with two Vietnamese PDCoV strains that were isolated in 2015. However, three Thai PDCoV strains and one Laotian PDCoV strain clustered in a new clade of PDCoVs that was separate from both the Chinese clade and the United States and South Korean clades.

In this study, we determined the full-length genome sequence of a PDCoV strain from Shanghai, China. A phylogenetic analysis showed that PDCoV strain SHJS/SL/2016 belongs to the Chinese clade, which might share a common evolutionary ancestor with the United States and South Korean clades, but it clustered separately from the Thai and Laotian PDCoV strains. These data will provide further insights into the epidemiology and evolution of PDCoV in China and facilitate investigations of the genetic diversity of PDCoV worldwide.

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References

1. Woo PC, Huang Y, Lau SK, Yuen KY (2010) Coronavirus genomics and bioinformatics analysis. Viruses 2: 1804-1820.
2. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, et al. (2012) Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol 86:3995-4008.
3. Wang L, Byrum B, Zhang Y (2014) Porcine coronavirus HKU15 detected in 9 US states. Emerg Infect Dis 20: 1594-1595.
4. Jung K, Hu H, Eyerly B, Lu Z, Chepngeno J, Saif LJ (2015) Pathogenicity of 2 porcine deltacoronavirus strains in gnotobiotic pigs. Emerg Infect Dis 21: 650-654.
5. Homwong N, Jarvis MC, Lam HC, Diaz A, Rovira A, et al. (2016) Characterization and evolution of porcine deltacoronavirus in the United States. Prev Vet Med 123: 168-174.
6. Li G, Chen Q, Harmon KM, Yoon KJ, Schwartz KJ, et al. (2014) Full-length genome sequence of porcine deltacoronavirus strain USA/IA/2014/8734. Genome announcements 2: e00278-14.
7. Marthaler D, Raymond L, Jiang Y, Collins J, Rossow K, et al. (2014) Rapid detection, complete genome sequencing, and phylogenetic analysis of porcinedeltacoronavirus. Emerg Infect Dis 20: 1347-1350.
8. ThePigSite (2014) Swine deltacoronavirus detected on Canadian pig farms.
9. Wang L, Byrum B, Zhang Y (2014) Porcine coronavirus HKU15 detected in 9 US states. Emerg Infect Dis 20: 1594-1595.
10. Wang L, Zhang Y, Byrum B (2014) Complete genome sequence of porcine coronavirus HKU15 strain IN2847 from the United States. Genome announcements 2: e00291-14.
11. Dong N, Fang L, Zeng S, Sun Q, Chen H, et al. (2015) Porcine deltacoronavirus in Mainland China. Emerg Infect Dis 21: 2254-2255.
12. Janetanakit T, Lumyai M, Bunpapong N, Boonyapisitsopa S, Chaiyawong S, et al. (2016) Porcinedeltacoronavirus, Thailand, 2015. Emerg Infect Dis 22: 757-759.
13. Lee S, Lee C (2014) Complete genome characterization of Korean porcine deltacoronavirus strain KOR/KNU14-04/2014. Genome announcements 2: e01191-14.
14. Lorsirigool A, Saeng-Chuto K, Temeeyasen G, Madapong A, Tripipat T, et al. (2016) The first detection and full-length genome sequence of porcine deltacoronavirus isolated in Lao PDR. Arch Virol 161: 2909-2911.
15. Madapong A, Saeng-Chuto K, Lorsirigool A, Temeeyasen G, Srijangwad A, et al. (2016) Complete genome sequence of porcine deltacoronavirus isolated in Thailand in 2015. Genome announcements 4: e00408-e00416.
16. Saeng-Chuto K, Lorsirigool A, Temeeyasen G, Vui DT, Stott CJ, et al. (2017) Different Lineage of Porcine Deltacoronavirus in Thailand, Vietnam and Lao PDR in 2015. Transbound Emerg Dis 64: 3-10.
17. Song D, Zhou X, Peng Q, Chen Y, Zhang F, et al. (2015) Newly emerged porcine deltacoronavirus associated with diarrhoea in swine in China: identification, prevalence and full-length genome sequence analysis. Transbound Emerg Dis 62: 575-580.
18. Jung K, Hu H, Eyerly B, Lu Z, Chepngeno J, et al. (2015) Pathogenicity of 2 porcine deltacoronavirus strains in gnotobiotic pigs. Emerg Infect Dis 21: 650-654.
19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731-2739.
20. Zhai SL, Wei WK, Li XP, Wen XH, Zhou X, et al. (2016) Occurrence and sequence analysis of porcine deltacoronaviruses in southern China. Virology Journal 13: 136.