Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short communication

Genetic characterization and phylogenetic analysis of porcine deltacoronavirus (PDCoV) in Shandong Province, China

Wenchao Sun\textsuperscript{a,b,1}, Li Wang\textsuperscript{b,1}, Haixin Huang\textsuperscript{a,1}, Wei Wang\textsuperscript{a}, Liang Cao\textsuperscript{c}, Jinyong Zhang\textsuperscript{c}, Min Zheng\textsuperscript{d}, Huijun Lu\textsuperscript{a,*}

\textsuperscript{a} Institute of Virology, Wenzhou University, Wenzhou, 325035, China
\textsuperscript{b} Shandong New Hope Liuhe Group Company, Qingdao, 266100, China
\textsuperscript{c} Institute of Military Veterinary Medicine, The Academy of Military Medical Sciences, Changchun, 130122, China
\textsuperscript{d} Guangxi Centre for Animal Disease Control and Prevention, Nanning, 530001, China

ARTICLE INFO

Keywords:
Porcine deltacoronavirus (PDCoV)
Spike protein
Nucleocapsid protein
ORF1a
Deletion

ABSTRACT

Porcine deltacoronavirus (PDCoV) is the etiological agent of acute diarrhoea and vomiting in pigs, threatening the swine industry worldwide. Although several PDCoV studies have been conducted in China, more sequence information is needed to understand the molecular characterization of PDCoV. In this study, the partial ORF1a, spike protein (S) and nucleocapsid protein (N) were sequenced from Shandong Province between 2017 and 2018. The sequencing results for the S protein from 10 PDCoV strains showed 96.7 %–99.7 % nucleotide sequence identity with the China lineage strains, while sharing a lower level of nucleotide sequence identity, ranging from 95.7 to 96.8%, with the Vietnam/Laos/Thailand lineage strains. N protein sequencing analysis showed that these strains showed nucleotide homologies of 97.3%–99.3% with the reference strains. Phylogenetic analyses based on S protein sequences showed that these PDCoV strains were classified into the China lineage. The discontinuous 2 + 3 aa deletions at 400–401 and 758–760 were found in the Nsp2 and Nsp3 coding region in five strains, respectively, with similar deletions having been identified in Vietnam, Thailand, and Laos. Three novel patterns of deletion were observed for the first time in the Nsp2 and Nsp3 regions. Importantly, those findings suggest that PDCoV may have undergone a high degree of variation since PDCoV was first detected in China.

1. Introduction

Porcine deltacoronavirus (PDCoV) was first discovered in 2009 in Hong Kong from swine fecal samples (Woo et al., 2012). PDCoV was recognized as the causative agent of acute diarrhoea and vomiting in pigs (Hu et al., 2015; Song et al., 2015; Wang et al., 2014). Since its appearance, PDCoV has caused significant economic losses for the swine industry worldwide. PDCoV is a member of the genus Deltacoronavirus in the family Coronaviridae and is an enveloped virus that has a positive-sense single-stranded RNA (+ssRNA) genome of 25.4 kb in length (Lee and Lee, 2015; Phan et al., 2018). The PDCoV genome has seven major open reading frames (ORFs). Two overlapping ORFs (ORF1a and ORF1b) encode two replication-associated proteins, which are both autoproteolytically cleaved into 15 nonstructural proteins (Nsp2 to Nsp16) (Wang et al., 2018a; Woo et al., 2010). The remaining ORF encodes the spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N). Additionally, three accessory proteins were identified: nonstructural protein 6 (NS6), NS7, and NS7a (Fang et al., 2017, 2016; Luo et al., 2016).

The S protein is the most variable protein among the PDCoV genes, with only 96.0 %–100 % amino acid sequence identity between Chinese and American strains (Zhang et al., 2019b). The S protein plays a pivotal role in the viral entry and stimulates the induction of neutralizing antibodies in the natural host (Chen et al., 2019c; Chiou et al., 2017; Lin et al., 2016; Zhang et al., 2015, 2014). PEDV N protein can antagonize beta interferon and interferon-λ production (Ding et al., 2014; Shan et al., 2018). SARS-CoV N protein can bind to DNA in vitro (Chen et al., 2007). bCoV-OC43 N protein interacts with the transcription factor nuclear factor-kappa B (NF-κB) (de Haan and Rottier, 2020).
The ORF1a region is the most variable region of the PDCoV genome and substitutions, deletions and insertions have been observed in the Nsp2 and Nsp3 coding region in Vietnam, Thailand, and Laos (Lorsirigool et al., 2017; Wang et al., 2015). To determine the molecular epidemiology and genetic variations of PDCoV in China, the partial ORF1a, S protein and N protein genes of 10 PDCoV strains from different pig farms located in Shandong Province were sequenced and analysed. This study may provide valuable information for the molecular epidemiology of PDCoV and its emerging variants in China.

2. Materials and methods

2.1. Sample collection

To monitor the prevalence and sequence properties of PDCoV in Shandong Province, China, a total of 58 porcine samples, including 21 faecal samples and 37 intestinal samples, were collected from different commercial swine from September 2017 to December 2018. All samples were stored at −80 °C and were subsequently used for RNA extraction.

2.2. RNA extraction and PDCoV detection

RNAs were extracted from the samples using the RNAeasy mini kit (TaKaRa BIO INC., Dalian, China) according to the manufacturer’s instructions. To detect, differentiate and sequence PDCoV, the One Step RT-PCR kit (TaKaRa Co., Dalian, China) was used to synthesize cDNA. Ten PDCoV-positive samples were selected for the partial ORF1a, S protein and N protein sequences. The primer sets are listed in Table 1.

2.3. Nucleotide sequencing and phylogenetic analysis

The partial ORF1a, S protein and N protein sequences of PDCoV were independently used for sequence alignments and phylogenetic analyses. The nucleotide and deduced amino acid sequences were

| Table 1 | List of primers used in the study. |
|---------|----------------------------------|
| Primer name | Primer sequence (5′–3′) | Size (bp) | Target Genes |
| PDCoV S1-F | 5′-ATGGAGAGGAGCTCTATTTGATATGAC-3′ | 1763 bp | S1 |
| PDCoV S1-R | 5′-AACTTGGCAAGTACTTGAACGGGAG-3′ | 1750 bp | S2 |
| PDCoV S2-F | 5′-ATTTTCTCTTCTGCTCAGAGCAGGAG-3′ | 1045 bp | N |
| PDCoV S2-R | 5′-CTAAGCCTGCTGATTCTTCTCTAT-3′ | 1290 bp | ORF1a/1b |

| Table 2 | PDCoV strains described in this study. |
|---------|----------------------------------|
| No. | Strain | Accession No. | Collection date | Collection Country | Length (bp) |
| 1 | HKU15-44 | JQ065042 | 2009 | China: Hong Kong | 25430 |
| 2 | USA/Iowa136/2015 | KX022602 | 2015 | USA: Iowa | 25382 |
| 3 | USA/Nebraska145/2015 | KX022605 | 2015 | USA: Nebraska | 25320 |
| 4 | USA/Michigan448/2014 | KR266580 | 2014 | USA: Michigan | 25394 |
| 5 | USA/Indiana453/2014 | KR266581 | 2014 | USA: Indiana | 25394 |
| 6 | USA/Nebraska210/2014 | KR266586 | 2014 | USA: Nebraska | 25404 |
| 7 | Ohio/CVM1/2014 | KJ692311 | 2014 | USA | 25433 |
| 8 | IL/2014/026PV_P11 | KP818395 | 2014 | USA: Illinois | 25422 |
| 9 | USA/Minnesota292/2014 | KR266584 | 2014 | USA: Minnesota | 25395 |
| 10 | YMG/JP/2014 | LC260044 | 2014 | Japan: Yamagata | 25362 |
| 11 | KNU14-04 | KM820765 | 2012 | South Korea | 25422 |
| 12 | P1_16_BTL_0115/PDCoV/2016/Lao | KX118627 | 2016 | Laos | 25405 |
| 13 | Vietnam/Binh21/2015 | KX834352 | 2015 | Vietnam: Binh | 25406 |
| 14 | Thailand/SS015L/2015 | KU051649 | 2015 | Thailand | 25405 |
| 15 | CH-SXD1/2015 | KT021234 | 2015 | China: Shangxi | 25419 |
| 16 | CH-Js-2014 | KP757892 | 2014 | China: Jiangsu | 25420 |
| 17 | CH/Sichuan/S27/2012 | KT266822 | 2012 | China: Sichuan | 25404 |
| 18 | CH-2004 | KP757890 | 2004 | China: Anhui | 25420 |
| 19 | CH-HB-2014 | KP757891 | 2014 | China: Hebei | 25420 |
| 20 | CH/Tianjin/2016 | KY065120 | 2016 | China: Tianjin | 25413 |
| 21 | CH/JXGS01/2016 | KY293077 | 2016 | China: Jiangxi | 25438 |
| 22 | CH/Hunan/2014 | KX513724 | 2014 | China: Hunan | 25413 |
| 23 | CH/Jiangsu/2014 | KYS13725 | 2014 | China: Jiangsu | 25422 |
| 24 | CH-HG-2017 | MF095123 | 2017 | China | 25399 |
| 25 | GD | MF431742 | 2015 | China: Guangdong | 25420 |
| 26 | SD | MF431743 | 2014 | China: Shandong | 25414 |
| 27 | CH/GS/2017 | MF642324 | 2017 | China: Guan | 25420 |
| 28 | CHN-IN-1601 | MG832584 | 2016 | China | 25419 |
| 29 | HNZK-02 | MH708123 | 2018 | China: Henan | 25453 |
Fig. 1. Phylogenetic analyses of PDCoV using the neighbour-joining algorithm and 1000 bootstrap replications in a heuristic search with 29 PDCoV reference strains available in GenBank. A S protein genomic sequence. B N protein sequence. C Partial ORF1a gene.
assessed using BioEdit 7.0. All the sequences were aligned using the MEGA5 program, and phylogenetic trees were constructed using the neighbour-joining method. The reliability of the branching orders was evaluated by the bootstrap test (n = 1,000). After nucleotide homology comparison and screening, 29 representative strains were selected from 102 strains for molecular evolutionary analyses (Tables 2 and S1). In addition, these strain sequences have been previously published as reference sequences (Suzuki et al., 2018; Zhang et al., 2019a, c; Zhang et al., 2019d).

3. Results

3.1. Sample screening and genome sequencing

Twelve of fifty-eight (20.68 %) field samples were positive for PDCoV, while the PEDV infection rate was 34.48 % (20/58), and the co-infection rate of PEDV and PDCoV was up to 50.00 % (10/20). 12 PDCoV positive samples were identified from 4 faecal samples and 8 intestinal samples. PRV was identified in 5 of 58 samples. Two of fifty-eight samples were found to be positive for TGEV. PRV/PEDV and TGEV/PEDV co-infections were 15.00 % (3/20) and 5.00 % (1/20), respectively. None of the PDCoV-positive samples were positive for TGEV and PRV. PDCoV/PEDV co-infections were the most common.

To obtain the sequence, the complete S protein, N protein and partial ORF1a genes were amplified in 10 PDCoV-positive samples (Table 1). The 10 strains were named SD01-2018, SD03-2018, SD05-2018, SD06-2018, SD07-2018, SD08-2018, SD09-2018, SD10-2018, SD11-2018, and SD12-2018. The GenBank accession numbers of the complete S protein, N protein and partial ORF1a genes were as follows: MN173803-MN173812, MN173783-MN173792, and MN173793-MN173802, respectively.

3.2. Phylogenetic analysis of the S, N and partial ORF1a/1b genes

3.2.1. Phylogenetic analysis of S protein

Phylogenetic analysis of the nucleotide sequences of the S protein sequences indicated that all strains worldwide can be categorized into three lineages: the China lineage, USA/Japan/South Korea lineage and Vietnam/Laos/Thailand lineage. All new PDCoVs strains from Shandong in our study belonged to the China lineage (Fig. 1A).
The sequence alignment results showed that these new strains shared nucleotide sequence homologies of 97.5%–99.9% with each other. They also shared up to 96.7%–99.7% nucleotide sequence similarity with the representative China lineage strains (HKU15-44, CHN-AH-2004, CHN-HN-2014 and CHN-HG-2017) and 97.5%–98.6% with the representative USA/Japan/South Korea lineage strains (OhioCVM1/2014, IL/2014/026PDV_P11, YMG/JPN/2014 and KNU14-04). By contrast, the new strains showed lower 95.7%–96.8% nucleotide sequence identity with the representative Vietnam/Laos/Thailand lineage strains (Vietnam/Binh21/2015, Thailand/S5011/2015 and P1_16_BTL_0115/2016/Lao).

3.2.2. Phylogenetic analysis of N protein

A phylogenetic tree was constructed using the N protein sequences from the strains isolated from Shandong and the references strains (Fig. 1B). SD07-2018, SD11-2018 and SD12-2018 with CHN-HG-2017) and 97.5% nucleotide identity with the China lineage strains (HKU15-44, CHN-AH-2004, CHN-HN-2014 and CHN-HG-2017). These strains shared 96.0% nucleotide identity with the China lineage strains (HKU15-44, CHN-AH-2004, CHN-HN-2014 and CHN-HG-2017). These strains shared 96.0% nucleotide identity with the USA/Japan/South Korea lineage strains and Viet Nam/Laos/Thailand lineage strains, respectively.

As shown in Fig. 2, all the PDCoV N protein sequences were the same length (1029 nt) and encoded 342 amino acid residues. Compared to other structural proteins, N protein is the most conserved structural proteins and remains the primary target protein for current diagnostic tests. The deduced amino acid identity values of N protein sequences were 98.4%–99.9% found among 10 strains. They shared 97.9–99.3%, 98.3%–98.9% and 99.9% nucleotide sequence identity with the China lineage strains, USA/Japan/South Korea lineage strains and Viet Nam/Laos/Thailand lineage strains, respectively.

3.2.3. Phylogenetic analysis of the partial ORF1a gene

A phylogenetic tree was generated based on the deduced aa sequence of the partial ORF1a gene. SD01-2018, SD03-2018, SD05-2018, and SD08-2018 strains were placed into one subgroup with the Vietnam/Laos/Thailand lineage strain and CH/Sichuan/S27/2012 strain. The other subgroup containing SD07-2018, SD09-2018, SD10-2018 and SD11-2018 was closer to the Chinese strain CHN-HG-2017. The SD12-2018 strain was placed into one subgroup (Fig. 1C).

The partial ORF1a gene of the 10 strains shared 95.9%–98.7% nucleotide identity with the China lineage strains (HKU15-44, CHN-AH-2004, CHN-HN-2014 and CHN-HG-2017). These strains shared 96.0%–97.5% nucleotide identity with the USA/Japan/South Korea lineage strains (OhioCVM1/2014, IL/2014/026PDV_P11, YMG/JPN/2014 and KNU14-04) and 95.3%–97.9% nucleotide identity with the Vietnam/Laos/Thailand lineage strains (Vietnam/Binh21/2015, Thailand/S5011/2015 and P1_16_BTL_0115/2016/Lao).

3.3. Amino acid variants in the spike proteins

Compared with the USA/Japan/South Korea lineage and Vietnam/Thailand lineage strains, the China lineage strains have 3-nt TAA deletions (52 N), leading to the lack of an asparagine in the S gene. This may be the most important feature of the Chinese lineage strains, except for CH/Jiangsu/2014 (Zhang et al., 2019d). Sequence analysis showed that 27 aa mutations were detected in the current diagnostic tests (McBride et al., 2014). Recently, a recombinant N protein-based indirect enzyme-linked immunosorbent assay (ELISA) (rPDCoV-N-ELISA) was established to detect PDCoV IgG antibodies (Su et al., 2016). Similar ELISA test has been done in Taiwan (Hsu et al., 2019). Compared with the reference strain HKU15-44, the following aa changes were detected in CHN-AH-2004, SD, P1_BTL_0115/2016/Lao and Thailand/S5011/2015 at 43 V/A, 163 S/P, and 265 I/M, respectively. Several amino acid mutations were detected in 10 strains at positions 18 L/P, 67 Y/T, 82 G/S, 215 G/A/F, 216 S/D, 222 G/A, 223 M/V, 291 P/S, 310 P/V, and 329 E/V. N protein is a multifunctional protein, the key conserved amino acid site mutations affect their function, for example, the Arg-76 and Tyr-94 residues in the IBV N protein are critical for RNA binding, SARS-CoV N protein also exists Arg-94 and Tyr-122 residues (Tan et al., 2006). PDCoV is difficult to isolate and there are few reverse genetic studies. So far, there have been no related reports that mutations in key amino acids of the N protein will affect the current diagnostic tests.
Among the 4 strains, SD07-2018, SD09-2018, SD11-2018 and SD12-2018 had another 2 unique substitutions at positions 513 F/S and 534C/N.

3.4. Novel amino acid deletions or insertions were detected in the partial ORF1a gene

The partial ORF1a gene has the highest genetic diversity in the genomes of the PDCoV strains (Xu et al., 2018). To determine whether the strains in this study possessed the characteristic deletions in the ORF1a gene found in the Thailand PDCoVs previously described, the partial ORF1a sequences containing the Nsp2 and Nsp3 hypervariable region (HVR) from 10 PDCoV strains were sequenced and aligned with the reference isolates, especially those with known pathogenicity, OhioCVM1/2014, IL/2014 /026PDV_P11, YMG/JPN/2014, and CHN-HG- 2017.

Compared with the OhioCVM1/2014, IL/2014 /026PDV_P11, YMG/JPN/2014, and CHN-HG-2017 strains, suggesting that these strains are the dominating strain circulating in Shandong Province. Four strains SD07-2018, SD09-2018, SD10-2018 and SD11-2018 in the partial ORF1a gene had a length of 1249 nucleotides, containing a continuous 0 + 7 amino acid deletion at position 758–761. The SD12-2018 strain in the Nsp3 gene had a length of 1261 nucleotides, and a 0 + 3 amino acid deletion at position 758–760. By contrast, no deletions or insertions were detected within the ORF1a gene in the Chinese strains collected early in the study. Additionally, the SD strain contained a 2 + 0 deletion at positions 400–401 in the Nsp2 gene.

As shown in Fig. 4, five strains (SD01-2018, SD03-2018, SD05-2018, and SD06-2018 and SD08-2018) with a 7-aa deletion in ORF1 regions had substitutions identical with those of the Thailand/S5015 L/2015 strain at positions 397A/S, 427I/L, 447A/T, 547 T/I, 550 N/D, 554 T/A, 599 F/L, 647A/V, 670 P/L, 717D/N, and 762 K/E. Additionally, five strains had unique substitutions at positions 529E/D, 778 P/S and 780 P/I. Notably, four strains (SD07-2018, SD09-2018, SD11-2018 and SD12-2018) carried substitutions at position 401 L/M.

4. Discussion

Several enteric coronaviruses have been identified from diarrheic piglets, such as porcine epidemic diarrhoea virus (PEDV) (Li et al., 2012), transmissible gastroenteritis virus (TGEV) (Xia et al., 2018),...
Swine acute diarrhea syndrome coronavirus (SADS-CoV), also named as Swine enteric alphacoronavirus (SeACoV) (Fu et al., 2018; Zhou et al., 2019) and PDCoV. Outbreaks of these enteric coronaviruses have been reported in China, causing substantial economic losses in the swine industry (Qing et al., 2016; Zeng et al., 2015; Zhou et al., 2019). In 2014, PDCoV was first detected in China, causing massive financial losses in the swine industry, and the virus has rapidly spread nationwide (Dong et al., 2015; Liu et al., 2018; Mai et al., 2018a; Song et al., 2015; Wang et al., 2018). Thus, the molecular characterization of these PDCoVs is a major focus of Chinese virological research.

Although several studies have shown an obvious increase in the genomic diversity of PDCoV in China (Dong et al., 2016; Liang et al., 2019; Mai et al., 2018b), previous studies have focused mainly on the S gene, while the genetics of the Chinese PDCoVs based on the ORF1 gene are not well characterized. The Vietnam/Laos/Thailand lineage strains had 6-nt (AGTTTG) and 9-nt (GAGCCAGTC) deletions in ORF1a (Le et al., 2018). In 2015, only a few strains with similar deletions have been reported in China, such as the CH/Sichuan/S27/2012 strain with a 5-nt (AGGTTG) deletion at position 400–401 and 7-nt (GAGCCAGTC) deletions in ORF1a (Wang et al., 2015). The ORF1a deletion region encoded Nsp2 and Nsp3 proteins. The Nsp3 protein acts as a scaffold protein to interact with itself and bind other viral Nsps or host proteins (Nogales et al., 2012; Yuan et al., 2015). Nsp3 protein contains one or two papain-like protease (PLpro) domain(s) with deubiquitinating (DUB) and deISGylating activities in SARS-CoV and MERS-CoV (Alfuvaires et al., 2017; Neuman, 2016). In MHV, the Nsp3 protein ubiquitin-like domains could interfere with pathways involving ubiquitinylated or ISGylated host targets, thereby leading to the disruption of host anti-viral signal transduction or protein degradation (Chen and Makino, 2004). Nsp3 protein was also recommended as a marker for monitoring coronavirus evolution and for surveying the molecular epidemiology in lineage C betaCoVs (Forni et al., 2016). Additionally, the MERS-CoV Nsp3 Arg911Cys mutation is recommended as a marker for monitoring coronavirus evolution and for surveying the molecular epidemiology in lineage C betaCoVs (Forni et al., 2016). Additionally, the MERS-CoV Nsp3 Arg911Cys mutation is an example of adaptive evolution (Shokri et al., 2019). In TGEV, Nsp3s 2, 3, and 8 were incorporated into the CoV virions, involving in CoV replication (Nogales et al., 2012).

Mutation and recombination are important mechanisms for PDCoV evolution. The USA/Japan/South Korea lineage strains were characterized by no discontinuous deletions in the Nsp2 and Nsp3 coding regions, while the Vietnam/Laos/Thailand lineage strains showed a discontinuous 5-aa deletion (2aa + 3aa) at 400–401 and 758–760 in the Nsp2 and Nsp3 coding regions. These regional deletions were also observed in a Chinese CH/Sichuan/S27/2012 strain. Here, we found 5 novel strains with the same deletion pattern of “2 + 3aa” in the Nsp2 and Nsp3 coding regions. Interestingly, the SD12-2018 and CHN-HG-2017 strains have a continuous “0 + 3aa” in the Nsp3 deletion at 758–760. Additionally, SD07-2018, SD09-2018, SD10-2018 and SD11-2018 strains have a continuous “0 + 7aa” deletion at 755–761 in Nsp3. Two continuous aa deletion in Nsp2 was also identified in a Chinese strain, SD, which has two “2 + 0aa” deletions at position 400–401. Remarkably, natural deletions and insertions occurred in the ORF1a sequence, and these have led to genome size differences among PDCoV strains. However, the role of double deletions in the ORF1 of this virus remains unclear. Thus, the current results indicate that the novel deletion of Nsp2 and Nsp3 may provide another approach to the diagnosis.

The PDCoV S protein is a glycoprotein of approximately 1383 amino acids with an apparent molecular mass of 180 kDa. Studies of genomic sequences analyses using S protein genes have shown that PDCoV can be further divided into three lineages: the China lineage, USA/Japan/South Korea lineage and Vietnam/Laos/Thailand lineage (Zhang et al., 2019d). The Vietnam/Laos/Thailand lineage has been detected in Southeast Asia (Jang et al., 2017; Nelson et al., 2019; Niederwerder and Hesse, 2018; Perez-Rivera et al., 2019; Suzuki et al., 2018). The China lineage has been the predominant lineage in China since 2014 (Li et al., 2018a; Zhang et al., 2019a). S protein-based phylogenetic trees showed that 7 strains belonging to the China lineage were classified into a minor branch with CHN-HG-2017. The evidence showed that the PDCoV strains of China have genetic diversity.

The PDCoV spike (S) protein comprises S1, a receptor-binding subunit, and S2, a membrane fusion subunit. The S1 domain is important for recognizing and binding to cell receptors. Additionally, the S1 domain contains several neutralizing epitopes that stimulate the induction of neutralizing antibodies. The S2 domain is involved in triggering the fusion of the viral envelope and target cell membrane.
Chen, J., Fang, P., Wang, M., Peng, Q., Ren, J., Wang, D., Peng, G., Fang, L., Xiao, S., Ding, Z., 2019b. Porcine deltacoronavirus nucleocapsid protein antagonizes IFN-beta production by impairing dsRNA and PACT binding to RIG-I. Virus Genes 55, 520–531.

Chen, P., Wang, K., Hou, Y., Li, H., Li, X., Yu, L., Jiang, Y., Gao, F., Yang, W., Yu, H., Yang, X., Fang, G., Zhou, Y., 2019. Genotyping and pathogenicity assessment of porcine epidemic diarrhea virus strains circulating in part of China during 2011–2017. Infect. Genet. Evol. 69, 153–165.

Chiu, H.Y., Huang, Y.L., Deng, M.C., Chang, C.Y., Jeng, C.R., Tsai, P.S., Yang, C., Pang, Y.P., Chang, H.W., 2015. Phylogenetic analysis of the 5′UTR (22 nucleotides) gene of the porcine epidemic diarrhea virus genome. Virus Res. 206, 196–203.

Choi, J., Jang, J., 2017. Porcine deltacoronavirus infects porcine foetal tissues and is transmitted through placenta. Emerg. Microbes Infect. 6, 1–9.

Choi, J., Jang, J., 2018. dRNA and PACT binding to RIG-I and LGP2 targets of porcine deltacoronavirus. Virus Genes 55, 520–531.

Chen, C.J., Makino, S., 2004. Murine coronavirus replication induces cell cycle arrest in interferon (IFN) (Chang et al., 2014; Liao et al., 2005). PDCoV N-regulating cyclin-CDK activity but also inhibits the synthesis of type-1 interferon (IFN). J. Virol. 79, 1196–1196.

Cheng, K., Chen, J., 2016. The N protein is the predominant antigen produced in coronavirus-infected cells, making it a major viral target. J. Virol. 90, 4265–4273.

Chen, J., Fang, P., Wang, M., Peng, Q., Ren, J., Wang, D., Peng, G., Fang, L., Xiao, S., Ding, Z., 2019a. Porcine deltacoronavirus nucleocapsid protein antagonizes IFN-beta production by impairing dsRNA and PACT binding to RIG-I. Virus Genes 55, 520–531.

Chen, P., Wang, K., Hou, Y., Li, H., Li, X., Yu, L., Jiang, Y., Gao, F., Tong, W., Yu, H., Yang, X., Fang, G., Zhou, Y., 2019. Genotyping and pathogenicity assessment of porcine epidemic diarrhea virus strains circulating in part of China during 2011–2017. Infect. Genet. Evol. 69, 153–165.

Chiu, H.Y., Huang, Y.L., Deng, M.C., Chang, C.Y., Jeng, C.R., Tsai, P.S., Yang, C., Pang, Y.P., Chang, H.W., 2015. Phylogenetic analysis of the 5′UTR (22 nucleotides) gene of the porcine epidemic diarrhea virus genome. Virus Res. 206, 196–203.

Choi, J., Jang, J., 2017. Porcine deltacoronavirus infects porcine foetal tissues and is transmitted through placenta. Emerg. Microbes Infect. 6, 1–9.

Chen, C.J., Makino, S., 2004. Murine coronavirus replication induces cell cycle arrest in interferon (IFN) (Chang et al., 2014; Liao et al., 2005). PDCoV N-regulating cyclin-CDK activity but also inhibits the synthesis of type-1 interferon (IFN). J. Virol. 79, 1196–1196.

Cheng, K., Chen, J., 2016. The N protein is the predominant antigen produced in coronavirus-infected cells, making it a major viral target. J. Virol. 90, 4265–4273.

Chen, J., Fang, P., Wang, M., Peng, Q., Ren, J., Wang, D., Peng, G., Fang, L., Xiao, S., Ding, Z., 2019a. Porcine deltacoronavirus nucleocapsid protein antagonizes IFN-beta production by impairing dsRNA and PACT binding to RIG-I. Virus Genes 55, 520–531.

Chen, J., Fang, P., Wang, M., Peng, Q., Ren, J., Wang, D., Peng, G., Fang, L., Xiao, S., Ding, Z., 2019b. Porcine deltacoronavirus nucleocapsid protein antagonizes IFN-beta production by impairing dsRNA and PACT binding to RIG-I. Virus Genes 55, 520–531.

Chen, P., Wang, K., Hou, Y., Li, H., Li, X., Yu, L., Jiang, Y., Gao, F., Yang, W., Yu, H., Yang, X., Fang, G., Zhou, Y., 2019. Genotyping and pathogenicity assessment of porcine epidemic diarrhea virus strains circulating in part of China during 2011–2017. Infect. Genet. Evol. 69, 153–165.
