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Discovery of a novel swine enteric alphacoronavirus (SeACoV) in southern China

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ABSTRACT

Outbreaks of diarrhea in newborn piglets without detection of transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV), have been recorded in a pig farm in southern China since February 2017. Isolation and propagation of the pathogen in cell culture resulted in discovery of a novel swine enteric alphacoronavirus (tentatively named SeACoV) related to the bat coronavirus HKU2 identified in the same region a decade ago. Specific fluorescence signal was detected in Vero cells infected with SeACoV by using a positive sow serum collected in the same farm, but not by using TGEV-, PEDV- or PDCoV-specific antibody. Electron microscopy observation demonstrated that the virus particle with surface projections was 100–120 nm in diameter. Complete genomic sequencing and analyses of SeACoV indicated that the extreme amino-terminal domain of the SeACoV spike (S) glycoprotein structurally similar to the domain 0 of the alphacoronavirus NL63, whereas the rest part of S structurally resembles domains B to D of the betacoronavirus. The SeACoV-S domain 0 associated with enteric tropism had an extremely high variability, harboring 75-amino-acid (aa) substitutions and a 2-aa insertion, compared to that of HKU2, which is likely responsible for the extended host range or cross-species transmission. The isolated virus was infectious in pigs when inoculated orally into 3-day-old newborn piglets, leading to clinical signs of diarrhea and fecal virus shedding. These results confirmed that it is a novel swine enteric coronavirus representing the fifth porcine coronavirus.

1. Introduction

Coronavirus (CoV) is an enveloped, single-stranded, positive-sense RNA virus of the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, which comprises four genera, *Alpha-*, *Beta-*, *Gamma-*, and *Delta-CoV*. CoVs infect humans, other mammals, and birds, causing subclinical or respiratory and gastrointestinal diseases (de Groot et al., 2011; Woo et al., 2012). As of date, three types of swine enteric CoVs (SeCoVs): transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV), have been identified to induce clinical diarrhea in young pigs (Jung et al., 2016; Pensaert and de Bouck, 1978). In particular, emergences of variant PEDV fatal to newborn piglets in China in late 2010 (Pan et al., 2012), and later in the United States in 2013 (Huang et al., 2013; Tian et al., 2014), have posed a serious threat to the pork industry. Most recently, several chimeric SeCoV strains with a TGEV genomic backbone replaced by a PEDV spike (S) gene were identified from swine fecal samples in Europe (Akimkin et al., 2016; Belsham et al., 2016; Boniotti et al., 2016), implying that novel SeCoV pathogens could emerge by inter-CoV recombination under co-infection. The S gene encodes a glycoprotein, forming trimer projections on the viral surface, which is a major structural protein critical for CoV attachment and entry into the host cell (Hulswit et al., 2016).

In addition to recombination events between two distinct CoVs, amino acid (aa) mutations in the S protein may alter the tropism of the virus. For example, 21-aa substitutions and a 7-aa insertion in the amino-terminal domain (NTD) of the S glycoprotein of a murine hepatitis CoV (MHV) variant confer the ability to bind and in some cases infect cells of nonmurine species including swine cells (Schickli et al., 2004). In this study, we report the isolation and genetic characterization of a novel swine enteric alphacoronavirus (tentatively named SeACoV), related to a bat enteric coronavirus, from a pig farm that reported newborn-piglet diarrhea in southern China in 2017. This is yet another example to corroborate that the extended host range of CoV,

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here from bat to pig, is likely associated with aa substitutions at the NTD of the S glycoprotein. Furthermore, we conducted a pilot experimental infection study with this novel SeACoV, confirming its infectivity and ability to induced clinical signs of diarrhea in piglets.

2. Materials and methods

2.1. Cell lines and cell cultures

Baby hamster kidney fibroblast cell line BHK-21 (ATCC CCL-10), swine testis cell line ST (ATCC CRL-1746), porcine kidney epithelial cell line LLC-PK1 (ATCC CL-101), and African green monkey kidney epithelial Vero cell (ATCC CCL-81) were individually grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin, streptomycin, w/v). A Vero cell line stably expressing the TGEV receptor porcine aminopeptidase N (Vero-pAPN) was cultured in DMEM supplemented with $10 \mu g/ml$ puromycin and antibiotics (unpublished data). All cells were grown at 37 °C with 5% CO_2.

2.2. RT-PCR detections

A pan-CoV RT-PCR assay was used to detect the unknown pathogen with a pair of primers: Cor-FW (5′-ACWCARHTVAAYYTNAARTAYGC-3′) and Cor-RV (5′-TCRCAYTTDGGRTARTCCCA-3′) as described (Moes et al., 2005). After the pathogen (SeACoV) was identified, specific primers targeting the SeACoV-nucleocapsid (N) gene (the forward primer SEAF: 5′-ATGGATAAACCTGAATGGAAGCG-3′, and the reverse primer SEAR: 5′-CACCATCTCAACCTCTTCCTCAG-3′) were used for virus detection during isolation and subsequent passages.

2.3. Virus isolation

Fecal specimens collected from diarrheic piglets and positive for SeACoV RNA were homogenized in DMEM containing antibiotics followed by centrifugation at $4000 \times g$ for 15 min. The supernatants was inoculated onto confluent monolayers of BHK-21, ST, LLC-PK1 or Vero cells cultured with the maintenance medium plus trypsin (MMT) at 37 °C and 5% CO_2. The MMT consisted of DMEM supplemented with 10% FBS, 1% antibiotics and $5 \mu g/ml$ trypsin (Sigma). Cells were observed daily to record the development of cytopathic effect (CPE) as described previously (Pan et al., 2012).

The virus strain isolated in Vero cells with MMT, designated as CH/GD-01/2017, was plaque-purified in the presence of trypsin using neutral red staining as described (Qin et al., 2017). It was passaged serially using the culture supernatant, and the viral titer was determined by plaque assay.

2.4. Electron microscopy

Supernatant from purified SeACoV-infected cell cultures showing CPEs was negatively-stained. Grids were stained with 2% sodium phosphotungstic acid (pH 6.8) for 1.5 min and examined using a Hitachi Model H-7650 TEM.

2.5. Immunofluorescence assay (IFA)

Vero cells infected with SeACoV on 24-well plates were washed twice with phosphate-buffered saline (PBS) and fixed with acetone. One hundred and fifty microliters of the collected sow serum samples at a 1:100 dilution in PBS was added to the cells in each well and incubated for 1 h at room temperature. Cells were washed thrice with PBS followed by addition of 150 μl FITC-labeled rabbit anti-pig IgG (Thermo Fisher Scientific, USA) at 1:500 dilution. After incubation for 1 h at room temperature, the cells were washed with PBS, stained with 150 μl 4′, 6-diamidino-2-phenylindole (DAPI) at 1:1000 dilution and

visualized under a fluorescence microscope.

For antibody cross-reactivity test, Vero cells infected with SeACoV or PEDV (ZJU/G2/2013 strain; GenBank accession no. KU558701), Vero-pAPN cells infected with TGEV (Purdue strain; a gift from Dr. Rong Ye at Shanghai Medical College of Fudan University), and LLC-PK1 cells infected with PDCoV (Hunan strain; GenBank accession no. KY513724) were stained with the anti-PEDV-N, anti-TGEV-N and anti-PDCoV-N monoclonal antibody (purchased from Medgene Labs, Brookings, SD, USA), respectively. The FITC-conjugated goat anti-mice IgG (Thermo Fisher Scientific, USA) was used as the secondary antibody followed by DAPI staining.

2.6. Genomic cloning and bioinformatics analyses

Total RNA was extracted from the isolated virus with TRIzol reagent, and cDNAs were subsequently amplified by SuperScript II with specific primers according to the manufacturer's instructions (Thermo Fisher Scientific). A total of 16 primer pairs based upon the bat CoV HKU2 strain GD430-2006 (GenBank accession no. EF203064; Supplemental Table S1) were designed to amplify the complete genome of SeACoV. PCR products were purified and cloned into a pCR-Blunt vector (Thermo Fisher Scientific). For each amplicon, three to five individual clones were sequenced to determine the consensus sequence. The sequences were assembled and analyzed using the DNASTAR program. Multiple alignments of the full-length genomes, non-structural protein genes and S genes with representative CoV sequences and phylogenetic analyses were performed using the neighbor-joining method in MEGA5.2, respectively. Structure homology-modeling of SeACoV S glycoprotein was performed by the SWISS-MODEL server (https://www.swissmodel.expasy.org/).

2.7. SeACoV infectivity study in neonatal piglets

A pilot animal experiment was approved by the Experimental Animal Ethics Committee of Zhejiang University (approval no. ZJU20170026). Briefly, ten 3-day-old conventional piglets, free of SeACoV, PEDV, TGEV, and PDCoV RNA in the feces, were assigned into two groups with 5 in each. Piglets in each group were housed with their mothers (SeACoV RNA and serum antibody negative as determined by IFA) without any artificially supplemental colostrum or milk. Piglets in group one were each challenged orally with a SeACoV/CH/GD-01/2017/P3 isolate at a dose of 1×10^5 plaque-forming units (PFU)/ml (3 ml per pig), whereas piglets in group two each received 3 ml of DMEM orally as negative controls. All the piglets were monitored daily for any signs of illness. Two piglets in each group were euthanized at 3 days post-infection (dpi) while the remaining three in each group were necropsied at 5 dpi. The duodenum, jejunum and ileum samples were subjected to histological examinations by hematoxylin and eosin (HE) staining, respectively. The villous height (VH) and the crypt depth (CD) were measured on a minimum of eight different sites per small intestinal segment, and the ratios of VH to CD were then calculated to quantify the villous atrophy according to previously described (Jung et al., 2014). Fecal swabs for viral RNA detection were collected at 0, 1, 2, 3, 4 and 5 dpi from all five pigs until they were alive.

3. Results and discussion

3.1. Emergence of a new coronavirus from diarrheal piglets in southern China, 2017

Beginning from February 2017, a remarkable increase in outbreaks of newborn-piglet diarrhea occurred in a commercial pig farm located in Guangdong province of southern China. Clinical signs of affected pigs were characterized by acute vomiting and watery diarrhea (Fig. 1A). The mortality rate was over 35% in piglets less than 10 days old during February–May 2017. In addition, the small intestine of the diseased pigs
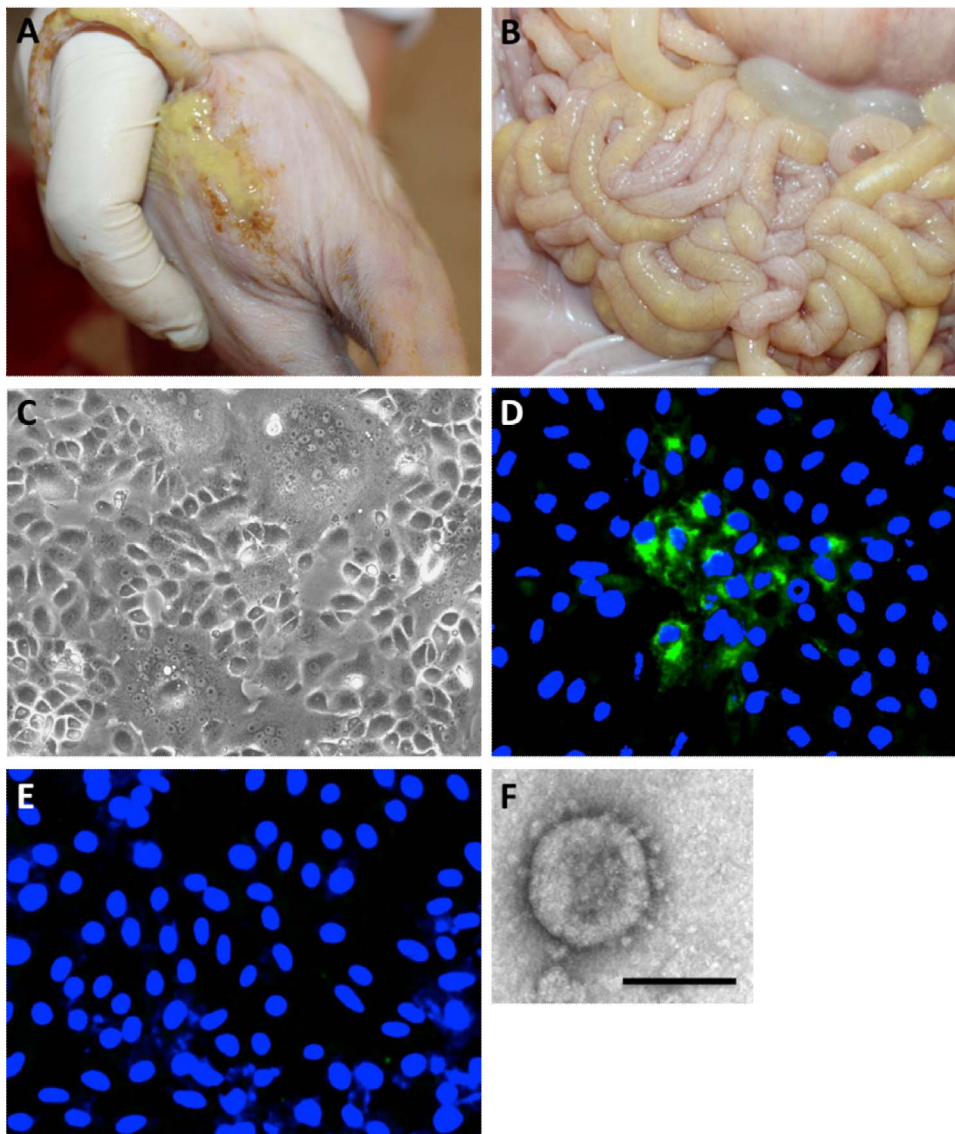
Fig. 1. Isolation and characterization of SeACoV. (A) A representative SeACoV-infected piglet showing clinical feature with watery feces. (B) Gross pathological lesions in the small intestine characterized by thin intestinal walls that contained moderate amounts of yellow watery feces. (C) Isolation of SeACoV-CH/GD-01/2017 in Vero cells showing CPE with syncytia formation at 48 h post-infection (hpi). (D and E) Immunofluorescence assay (IFA) result of SeACoV-infected Vero cells at 24 hpi (magnification = 200×). A positive sow serum sample (D) or a negative serum sample (E) (1:100 dilution each) collected from the disease outbreak farm was used as the first antibody followed by staining with a FITC-labeled rabbit anti-pig IgG. (F) Electron microscope image of the SeACoV/CH/GD-01 isolate (passage 5 on Vero cells) using phosphotungstic acid negative staining. Bar = 100 nm. All the results showed in panels C to F were obtained from plaque-purified virus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

displayed thin walls and contained yellow watery feces (Fig. 1B), which was indistinguishable from that of PEDV infection described previously (Huang et al., 2013; Pan et al., 2012). Fecal and small intestinal samples collected from affected piglets in this farm were submitted to our labs at Zhejiang University and Hog Production Division of Wen's Foodstuffs Group, respectively, for routine laboratory diagnostics. Upon laboratory analysis by RT-PCR, RNA of PEDV, TGEV, PDCoV or porcine he-magglutinating encephalomyelitis virus (PHEV), was not detected in these samples (data not shown). Other possibly known viral pathogens associated with piglet diarrhea such as porcine enterovirus, rotavirus or mammalian orthoreovirus (Qin et al., 2017) also could not be detected.

Subsequently, samples were tested by a pan-CoV RT-PCR assay designed to amplify a conserved region of 251-bp in the ORF1b gene for all CoV members (Moes et al., 2005). This test was positive for all the selected samples collected during February to May (data not shown). Sequencing of the PCR products revealed that they were 100% identical to the corresponding region (nucleotide [nt] positions 14024-14274) of four known bat enteric alphacoronavirus HKU2 strains (GenBank accession nos. EF203064 to EF203067) identified from Guangdong province and Hong Kong in 2004 and 2006 (Lau et al., 2007). The prevalence rate of bat CoV HKU2 from these two regions was reported to be 10.9% (7/64) and 8.3% (29/348) in Chinese horseshoe bats (*Rhinolophus sinicus*), respectively (Lau et al., 2007). HKU2 infection

associated with the other animal species has never been investigated. The results from pan-CoV RT-PCR detection indicated that an HKU2-like viral pathogen might be responsible for outbreaks of diarrhea in the pig farm.

3.2. Isolation and characterization of SeACoV in cell culture

In an effort to isolate the novel swine enteric HKU2-related CoV (SeACoV), suspension supernatants of selected HKU2-positive samples were prepared and inoculated in a panel of BHK-21, ST, LLC-PK1 and Vero cell lines routinely used to isolate porcine CoVs. Cultured supernatants from each inoculated cell line were blind-passaged serially. From Vero cell culture, we successfully isolated one SeACoV strain with CPE characterized by syncytia formation at 48 h post-infection, beginning from passage two (P2) and in the following passages after plaque purification (Fig. 1C). Furthermore, viral antigens were demonstrated in SeACoV-infected Vero cells by IFA, with a serum sample collected from a sow mothering the diseased piglets (Fig. 1D), but not with the specific monoclonal antibodies against the N protein of PEDV, TGEV or PDCoV (Fig. 2), suggesting that SeACoV are probably antigenetically distinct from the three known porcine CoVs. SeACoV antibody-negative sera from the same farm were also found, as staining with these sera in SeACoV-infected cells displayed no fluorescent signal (Fig. 1E).
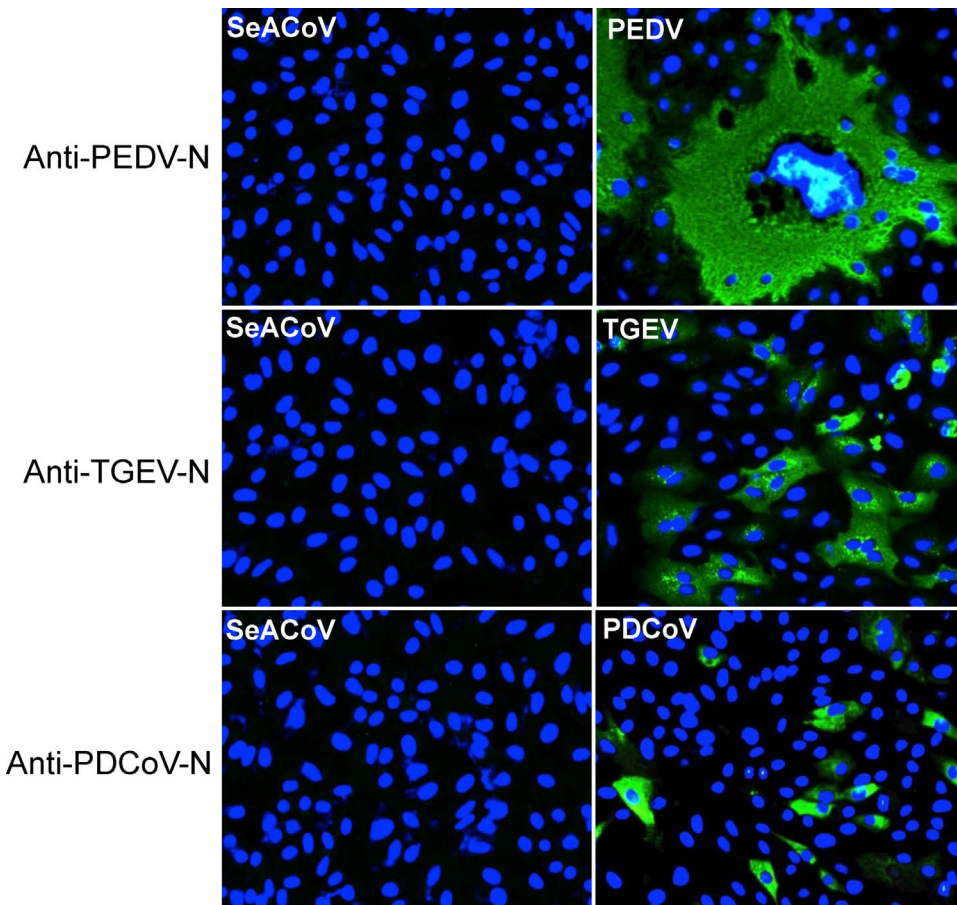
Fig. 2. IFA results of Vero cells infected with SeACoV or PEDV, Vero cells stably expressing porcine aminopeptidase N (Vero-pAPN) infected with TGEV, and LLC-PK1 cells infected with PDCoV at 48 h post-infection. SeACoV-infected Vero cells were stained with the anti-PEDV-N, anti-TGEV-N or anti-PDCoV-N monoclonal antibody, respectively (left panels). Cells infected with PEDV, TGEV or PDCoV were stained with the respective virus-specific antibody as the controls (right panels). The FITC-conjugated goat anti-mice IgG was used as the secondary Ab in IFA. Magnification = 200×.

Electron microscopy of a negatively stained sample from the supernatant of virus-infected Vero cells demonstrated that the virus particle was 100 to 120 nm in diameter, and had surface projections typical of CoV (Fig. 1F). SeACoV RNA was detected in supernatants from all virus passages to date (P2 to P8) by RT-PCR with primers SEAF and SEAR. The virus titer reached up to 1×10^6 PFU/ml at P8. This isolated CoV strain was designated as SeACoV/CH/GD-01/2017.

3.3. Complete genomic sequence of the emergent SeACoV revealed its potential origin and unique genetic features in the S glycoprotein

We next determined the complete genome of P2 of CH/GD-01 strain by RT-PCR amplification of 16 regions covering the entire SeACoV, as described previously for PEDV or PDCoV genomic cloning (Huang et al., 2013; Wang et al., 2015b). The complete genome sequence of the CH/GD-01/2017/P2 strain has been deposited in GenBank under accession no. MF370205.

The genomic sequence of CH/GD-01/2017/P2 is 27,155 nt in length, excluding the poly(A) tail. The genome organization is similar to those of the four HKU2 strains and a bat CoV identified in Yunnan province in southwestern China (BtRf-AlphaCoV/YN2012, GenBank no.

KJ473808), with the typical gene order 5′-ORF1a/1b (ORF1ab)-S-ORF3-E-M-N-NS7a-3′ (Fig. 3). The CH/GD-01/2017/P2 strain is 6-nt longer than HKU2 (27,149 nt), including a 3-nt (TTG) insertion at nt 4,554-4,555 (corresponding to the HKU2/GD430 sequence) in the nonstructural protein (nsp) 3 region, a 6-nt (GGCCTC) insertion at nt 20,504-20,505 in the S gene, and a 3-nt (GTA) deletion at nt 24,772-24,775 in the M gene (Fig. 3). However, these insertions/deletion are not unique for SeACoV since they are also present in the BtRf-AlphaCoV/YN2012 genome in comparison with HKU2.

SeACoV shared 94.9% nt sequence identity with the four HKU2 strains, and exhibited 88.3% nt identity with BtRf-AlphaCoV/YN2012. Accordingly, SeACoV is phylogenetically located between HKU2 and BtRf-AlphaCoV/YN2012, together forming a sublineage closely related to the proposed alphaCoV group-1b lineage, including PEDV and human CoVs NL63 and 229E, at the complete genome level (Fig. 4A).

However, analysis of the phylogenetic tree constructed based on the S genes (Fig. 4B) indicated that these six HKU2-related CoV strains along with a newly identified rat alphaCoV, LRNV (Wang et al., 2015a), formed a separate lineage clustered within the betaCoVs. The previous studies have suggested that HKU2 and the related LRNV probably resulted from an ancient recombination event with an alphaCoV genomic
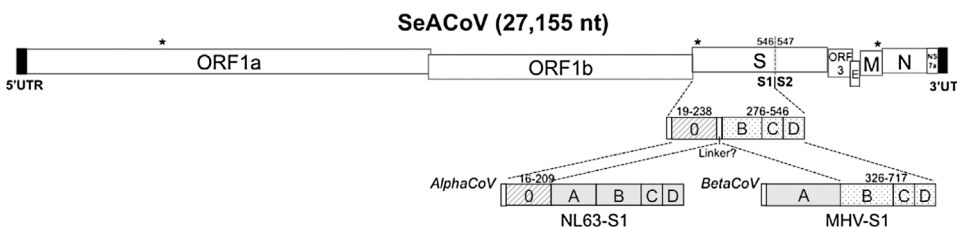

Fig. 3. Schematic diagram of the genomic structure of SeACoV and the proposed domain organization of the SeACoV spike protein S1 subunits according to the structure similarity analysis with NL63 and MHV that are both structure available. Numbers indicate amino acid positions in S glycoprotein of SeACoV, NL63 or MHV, respectively. See Supplemental Fig. S1 for the detailed sequence alignment. Nucleotide insertion/deletion at three locations (nsp3, S and M genes) in SeACoV compared to the consensus sequences of four bat-CoV HKU2 strains (GenBank accession nos. EF203064 to EF203067) are marked by "*".
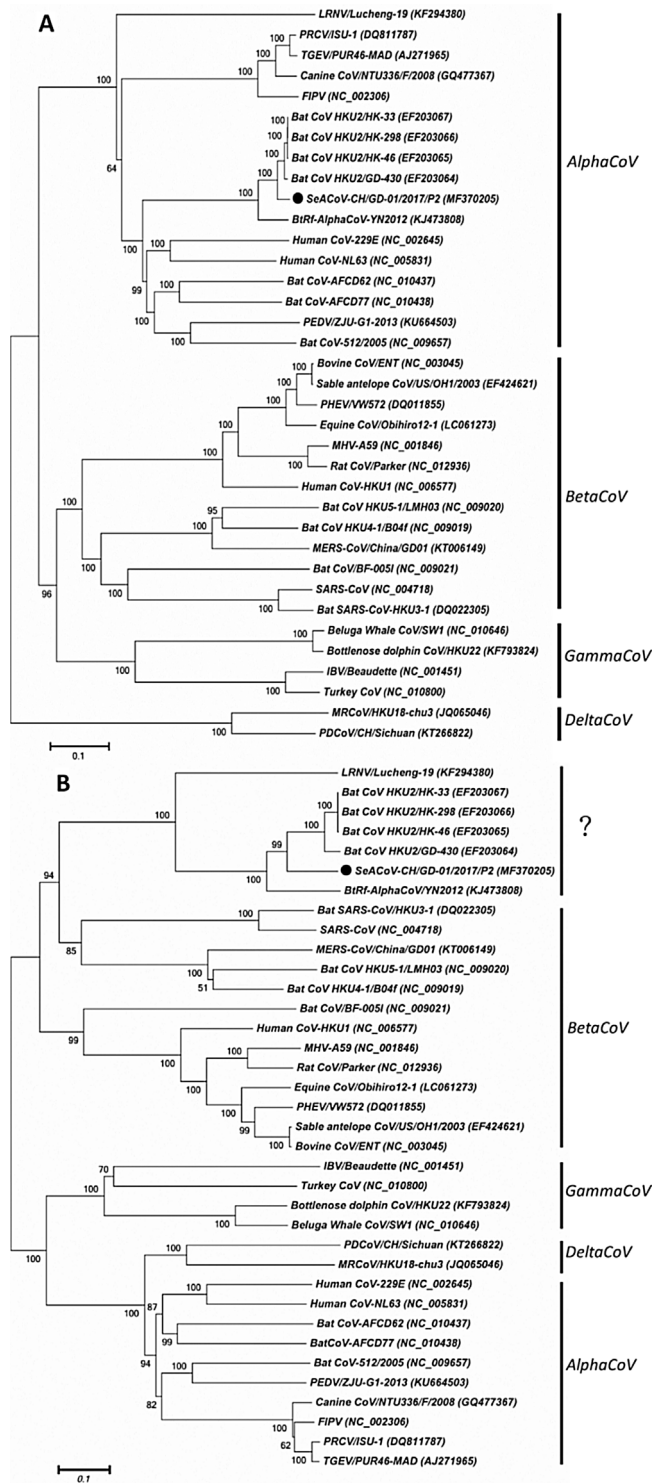
Fig. 4. Phylogenetic analysis of SeACoV (GenBank accession no. MF370205) and the other representative coronaviruses based upon nucleotide sequences of the full-length genome (A) or the spike gene (B). The trees were constructed by the neighbor-joining method. Bootstrap values are indicated for each node from 1000 resamplings. The names of the viruses and strains as well as GenBank accession numbers, are shown. The black solid circle indicates the new SeACoV reported in this study.

backbone replaced by a betaCoV S gene ([Lau et al., 2007; Wang et al., 2015a](#)). Furthermore, pairwise comparison of SeACoV genomic sequence with HKU2 indicated that the most dissimilar region was in the S gene, particularly, in the extreme NTD (aa 1-238). The entire SeACoV S protein had 86.4% aa identical with S of the HKU2/GD430 strain, but there was only a 67.4% identity in the extreme NTD of the S protein (S-

NTD) between SeACoV and HKU2. We identified a total of 75-aa substitutions plus a 2-aa insertion (resulting from a 6-nt insertion as mentioned above) within the SeACoV S-NTD compared to HKU2. In contrast, only 78 aa substitutions were found in the remaining part of the S protein. The extreme NTD changes in SeACoV are likely to be associated with the extended host range, similar to a previously reported MHV variant that was able to expand nonmurine-species tropism, with the phenotype mapped to 21 substitutions and a 7-aa insert in NTD of S1 subunit ([Schickli et al., 2004](#)).

During the time of this manuscript preparation, a sequence of another HKU2-related SeACoV strain GDS04, identified in the same region, was reported online but it did not give in-depth analyses ([Gong et al., 2017](#)). It remains unknown if GDS04 can be isolated in cell culture. Moreover, neither detection of serum anti-SeACoV antibodies nor observation of virus morphology was demonstrated. Nevertheless, comparative sequence analysis showed that the GDS04 strain, having the same genomic size (27,155 nt), shared 99.8% nt homology with GD-01/2017/P2 at the complete genome level. However, the GDS04 sequence was determined by the next generation sequencing, which should theoretically be less accurate than the GD-01/2017/P2 sequence determined based upon the consensus sequences from different short RT-PCR fragments covering the full-length genome. The S-NTD of GDS04 also contains 75-aa substitutions and a 2-aa insertion compared to that of HKU2. There are only three aa differences at the positions 86 (D/G), 166 (M/R) and 208 (A/V) in the S-NTD between GDS04 and GD-01/2017/P2. The corresponding aa in bat CoV HKU2 at these positions are G, M and M. For nonstructural protein genes analysis, the SeACoV GD-01/2017/P2 exhibited 99.8% and 97.7% nt identities, 99.8% and 97.5% nt identities, or 99.9% and 98.0% nt identities with GDS04 and HKU2 based on the ORF1ab, the ORF1a, or the ORF1b genes, respectively. These sequence analyses suggested that the SeACoV strains GD-01/2017 and GDS04 could have the same origin.

The S glycoprotein of SeACoV or HKU2 is unique and not related to any currently known betaCoVs at the aa sequence level. Most recently, the structures of several CoV S glycoprotein trimmers have been resolved ([Walls et al., 2016a, 2016b](#)). The betaCoVs comprise of four domains (domains A-D) in the S1 subunit whereas human alphaCoV NL63 shows an additional domain, named domain 0 (equivalent to the extreme NTD) compared to betaCoVs ([Fig. 3](#)). Therefore, a structure homology-modeling was performed in order to better understand the evolutionary origin of the S glycoprotein of SeACoV/HKU2 in the protein structure level. Surprisingly, the result suggested a hybrid structure of SeACoV-S: the extreme NTD (domain 0) of SeACoV-S is structurally similar to that of NL63, whereas the rest part in S1 structurally resembles domains B to D of the betaCoV MHV ([Fig. 3](#) and Supplemental Fig. S1). The S2 subunits of SeACoV and MHV also have a similar structure (Supplemental Fig. S1). The deduced structure of the linking region between domain 0 and domain B of SeACoV is uncertain. We hypothesize that the domain A is likely not present, which may be a unique feature of SeACoV/HKU2 S1 subunit. Since domain 0 and domain A are structurally similar and might come from a gene-duplication event ([Walls et al., 2016b](#)), we also hypothesize that either of them is likely dispensable in the S1 of CoVs. In addition, the presence of domain 0 in SeACoV/HKU2 is in line with the enteric tropism of these viruses since PEDV and TGEV also possess this domain ([Hulswit et al., 2016](#)). Future study on developing the SeACoV infectious clone and resolving the alphaCoV/betaCoV-hybrid SeACoV-S glycoprotein structure are warranted to confirm these findings.

3.4. SeACoV was infectious and pathogenic in piglets in experimental infection

In order to test whether or not, SeACoV is able to infect pigs, we performed a pilot challenge experiment using the cell-cultured SeACoV/CH/GD-01/2017 isolate. As expected, the five piglets in DMEM-inoculated group neither showed clinical sign nor shedding
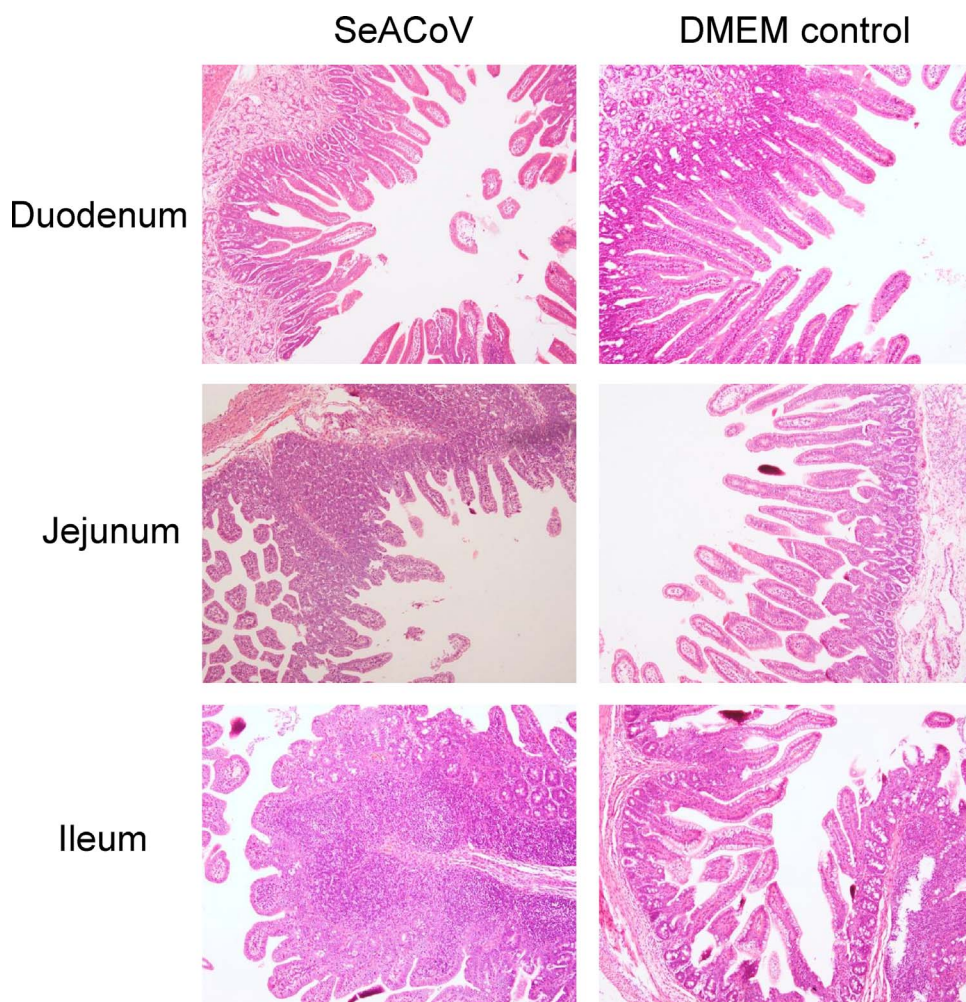
SeACoV DMEM control

Duodenum

Jejunum

Ileum

Fig. 5. Representative histological examinations of the duodenum, jejunum and ileum samples collected at 3 days post-infection from piglets inoculated with SeACoV or DMEM in the animal challenge experiment. Sections of jejunum and ileum in the SeACoV-infected group showed scattered areas of villi atrophy, whereas the section of duodenum showed mild microscopic lesions as compared to the DMEM control group.

virus in the feces throughout the experimental period (data not shown). In contrast, clinical signs characterized by acute vomiting and watery diarrhea (similar to Fig. 1A) were observed in the five SeACoV-infected piglets at 27 to 40 h post-infection, and thereafter lasted until necropsy. Fecal virus shedding was detected in five SeACoV-infected pigs at 1, 2 and 3 dpi, and in three remaining pigs at 4 and 5 dpi by RT-PCR with the primers SEAF and SEAR (data not shown). Sequencing of the PCR products indicated that they were identical with the SeACoV N gene sequence, confirming that the infectious virus was originated from the SeACoV isolate.

Upon histopathological analysis, no intestinal lesions were observed in control pigs (Fig. 5); the mean duodenal, jejunal and ileal VH/CD were 3.58 (± 0.82), 6.14 (± 1.55) and 4.70 (± 1.73), respectively. Typical microscopic lesions, showing gradual atrophy with significantly reduced VH/CD (the mean jejunal or ileal value was 2.26 [± 0.44] or 0.65 [± 0.37]), diminishing capillaries and central lacteals of the intestinal villous (Fig. 5), were detected in the jejunum and ileum of SeACoV-infected piglets. The duodenal sections displayed only mild microscopic lesions (the mean duodenal VH/CD = 2.59 [± 0.27]) in all SeACoV-infected pigs (Fig. 5). It was different from the result observed for the experimental infection using the virulent Chinese PEDV strain, in which marked microscopic lesions in all the three parts of the small intestine were found (Zhang et al., 2015). The results indicated that the SeACoV isolate is actually infectious and causes diarrhea in pigs. Since the specific non-swine antibodies against the structural proteins of SeACoV are not available currently, further comprehensively pathological studies by immunohistochemistry and serological assays, which is not the scope of this study, are warranted to provide more information on SeACoV infection.

4. Conclusion

In summary, we have isolated, sequenced and genetically characterized a novel swine enteric alphacoronavirus, which is probably distinct from PEDV, TGEV and PDCoV antigenetically, from diarrheal samples in a pig farm of southern China in 2017. The isolated SeACoV can actually infect and cause diarrhea in pigs, and should represent the fifth porcine coronavirus in addition to PEDV, TGEV (considering that porcine respiratory virus, PRCV, is a variant of TGEV), PDCoV and PHEV. To our knowledge, this is also the first study describing SeACoV related to the bat coronavirus HKU2 that could be isolated and propagated in cell culture. However, infection of Vero cells (a monkey cell line) with SeACoV also raises concerns about its potential host range other than swine. We also identified that the extreme NTD (aa 1-238) of SeACoV spike protein consists of 75-aa substitutions and a 2-aa insertion compared to that of HKU2, which is likely to be responsible for the cross-species transmission. Moreover, this region but not the other betaCoV-related domains of SeACoV S1 subunit is structurally similar to the alphaCoV domain 0, implying that these viruses gained enteric tropism through this domain. The results provide much needed information on SeACoV and HKU2 evolution, and the availability of SeACoV in cell culture will guide future efforts to develop effective vaccines against SeACoV.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/http://dx.doi.org/10.1016/j.vetmic.2017.09.020.

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