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Short Communication

Structural alteration of tight and adherens junctions in villous and crypt epithelium of the small and large intestine of conventional nursing piglets infected with porcine epidemic diarrhea virus



Kwonil Jung^{*}, Bryan Eyerly, Thavamathi Annamalai, Zhongyan Lu, Linda J. Saif^{**}

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural, and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA

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ABSTRACT

Integrity of the intestinal epithelium is critical for proper functioning of the barrier that regulates absorption of water and restricts uptake of luminal bacteria. It is maintained mainly by tight junctions (TJs) and adherens junctions (AJs). We conducted immunofluorescence (IF) staining for *in situ* identification of zonula occludin (ZO)-1 proteins for TJ and E-Cadherin proteins for AJ in the small and large intestinal villous and crypt epithelium of nursing pigs infected with porcine epidemic diarrhea virus (PEDV). Twenty 9-day-old piglets [PEDV-infected ($n=9$) and Mock ($n=11$)] from PEDV seronegative sows, were orally inoculated [8.9 log₁₀ genomic equivalents/pig] with PEDV PC21A strain or mock. At post-inoculation days (PIDs) 1–5, infected pigs showed severe watery diarrhea and/or vomiting and severe atrophic enteritis. By immunohistochemistry, PEDV antigens were evident in enterocytes lining the villous epithelium. At PIDs 1–5, PEDV-infected pigs exhibited mildly to extensively disorganized, irregular distribution and reduced expression of ZO-1 or E-Cadherin in villous, but not crypt epithelial cells of the jejunum and ileum, but not in the large intestine, when compared to the negative controls. The structural destruction and disorganization of TJ and AJ were extensive in PEDV-infected pigs at PIDs 1–3, but then appeared to reversibly recover at PID 5, as evident by increased numbers of ZO-1-positive epithelial cells and markedly improved appearance of E-Cadherin-positive villous epithelium. Our results suggest a possible involvement of structurally impaired TJ and AJ in the pathogenesis of PEDV, potentially leading to secondary bacterial infections.

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^{*} Corresponding author at: Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural, and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA. Tel.: +1 330 202 3569; fax: +1 330 263 3677.

^{**} Corresponding author at: Food Animal Health Research Program, Ohio Agricultural Research and Development Center, College of Food, Agricultural, and Environmental Sciences, Department of Veterinary Preventive Medicine, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA. Tel.: +1 330 263 3744; fax: +1 330 263 3677.

E-mail addresses: jung.221@osu.edu (K. Jung), saif.2@osu.edu (L.J. Saif).

1. Introduction

The intestinal epithelium provides a robust barrier that regulates absorption of nutrients and water and also restricts uptake of luminal bacteria (Su et al., 2011). The integrity of the intestinal epithelium is maintained by tight junctions (TJs), adherens junctions (AJs), desmosomes, and gap junctions. The TJ is the major paracellular barrier and functions to separate apical and basolateral compartments or membranes. The AJ forms a continuous belt between cells and is crucial for the maintenance of intercellular adhesion (Hartsock and Nelson, 2008; Su et al., 2011). The TJ and AJ are regulated by transmembrane TJ (occludin and claudin) and AJ (E-Cadherin and catenins) proteins on the opposing cells' plasma membranes and interactions mediated by actin binding proteins such as the zonula occludin (ZO) family, which link the AJ complex to actin microfilaments (Hartsock and Nelson, 2008; Su et al., 2011).

Porcine epidemic diarrhea virus (PEDV), which belongs to the genus *Alphacoronavirus* in the family *Coronaviridae*, causes high mortality of suckling pigs and substantial economic losses (Saif et al., 2012). Epidemic PEDV strains are highly enteropathogenic and acutely infect villous epithelial cells of the entire small and large intestines, but the jejunum and ileum are the primary sites of infection (Jung and Saif, 2015; Jung et al., 2014). During the early stages of PEDV infection, necrosis and exfoliation of infected villous epithelial cells is pronounced, resulting in acute, severe villous atrophy. To what extent the integrity and function of the PEDV-infected villous epithelium is restored by intestinal stem cells located at crypt cell layers is currently unclear. The aim of the present study was to determine whether PEDV infection causes structurally altered TJ and AJ in the villous and crypt epithelium of the small and large intestine by immunofluorescence (IF) staining for *in situ* identification of the related proteins, ZO-1 and E-Cadherin for TJ and AJ, respectively.

2. Materials and methods

2.1. Virus

The wild-type US PEDV strain PC21A was obtained from the intestinal contents of a diarrheic 1-day-old piglet on an Ohio farm in June 2013 (Jung et al., 2014). The original sample was serially passaged 2 times in Gn pigs. The original sample and Gn pig-passaged PC21A strain were confirmed to contain only PEDV, as reported in our previous study (Jung et al., 2014). The titer of Gn pig second-passaged PC21A strain was $11.8 \log_{10}$ GE/ml and was used as virus inoculum after dilution in minimal essential medium (MEM).

2.2. Conventional specific-pathogen-free pigs and experimental pig infection

Two seronegative, Large White \times Duroc crossbred, pregnant sows to acquire 20 nursing piglets, were obtained from a PEDV-free SPF (confirmed by history and seronegative sows; lack of qRT-PCR positive-fecal samples) swine

herd of The Ohio State University. The SPF herd was seronegative for antibodies to PRRSV, PRCV, TGEV and porcine circovirus type 2. Twenty 9-day-old nursing piglets were randomly assigned to one of two groups: PEDV inoculated ($n=9$) and Mock ($n=11$). Each experimental group of pigs was housed in a separate room in a high-security isolation facility (biosafety level 2). Nursing pigs were inoculated orally [$8.9 \log_{10}$ GE ($\approx 2.9 \log_{10}$ plaque forming units)/pig] (Jung et al., 2014) with 1 ml of PC21A or mock inoculated with MEM. Inoculated and negative control pigs ($n=3-4$ /group at each time-point) were euthanized for pathological examination at an acute-stage (PID 1), at a mid-stage (PID 3), and at a later-stage (PID 5) of infection. After PEDV inoculation, the pigs were monitored for clinical signs 2–3 times daily until necropsy. The Institutional Animal Care and Use Committee (IACUC) of the Ohio State University approved all protocols related to the animal experiments and care in this study.

2.3. Gross and histological analysis and immunohistochemistry for the detection of PEDV antigen

Small (duodenum, proximal, middle and distal jejunum, and ileum) and large (cecum/colon) intestinal tissues and other major organs (lung, liver, heart, kidney, spleen, and lymph node) were examined grossly and histologically at PIDs 1, 3, and 5. Tissues were placed in 10% phosphate buffered formaldehyde (pH 7.0), dehydrated in graded alcohol, embedded in paraffin, and cut in 3- μ m sections onto microscope slides, fixed and stained with hematoxylin and eosin (H&E) then analyzed for histopathological changes. Villous height and crypt depth were estimated by measuring at least 10 villi and crypts throughout the section. Mean ratios of jejunal villous height to crypt depth (VH:CD) were calculated as previously described (Jung et al., 2006). The formalin-fixed, paraffin-embedded tissues were prepared and tested by immunohistochemistry (IHC) for the detection of PEDV antigens, using monoclonal antibody 6C8-1 against the spike protein of PEDV strain DR13 (provided by Dr. Daesub Song, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea). The antibody was diluted 1:200 in PBST [phosphate-buffered saline (PBS) containing Tween 20, 0.1%]. IHC was conducted, as described previously (Jung et al., 2009).

2.4. Immunofluorescence (IF) staining for the detection of ZO-1 and E-Cadherin

The frozen jejunal/ileal and cecal/colonic tissues were prepared in Tissue-Tek OCT compound (Sakura, Torrance, CA, USA) and tested by IF staining for the detection of ZO-1 and E-Cadherin using monoclonal antibodies against human recombinant ZO-1 and human E-Cadherin (Invitrogen, CA, USA). The anti-ZO-1 antibody was diluted 1:100 in PBST and incubated on the tissues at 4 °C overnight. The anti-E-Cadherin antibody was diluted 1:100 in PBST and incubated on the tissues at 4 °C overnight, and an anti-mouse antibody conjugated with Alexa Fluor[®] 488 (Invitrogen) was used as the detection antibody and incubated on the tissues at 37 °C for 1 h. The

stained tissues were examined by fluorescence microscopy. ZO-1- or E-Cadherin-positive scores were computed by estimating the number of IF-positive cells in the intestinal section per microscopic area, at $\times 400$ magnification based on the following criteria: 0, no positive cells; 1, 1–29% of ZO-1- or E-Cadherin-positive villous or crypt epithelium showed staining; 2, 30–69% of ZO-1- or E-Cadherin-positive villous or crypt epithelium showed staining; and 3, 70–100% of ZO-1- or E-Cadherin-positive villous or crypt epithelium showed staining. Because the entire intestinal villous and crypt epithelium of uninfected and PEDV-infected pigs (E-Cadherin-positive scores, all 3) were positive for E-Cadherin, and E-Cadherin-stained villous epithelium (but not crypt epithelium) showed evident disorganization, E-Cadherin-stained tissues were additionally scored by the following system: 0, no positive cells; 1, 1–29% of E-Cadherin-positive villous epithelium showed disorganization; 2, 30–69% of E-Cadherin-positive villous epithelium showed disorganization; and 3, 70–100% of E-Cadherin-positive villous epithelium showed disorganization. Disorganization was characterized by irregular distribution and mildly to moderately reduced expression of E-Cadherin in villous epithelial cells of the small intestine.

2.5. Data analysis

All values are expressed as the means \pm standard deviation of the means (SDM). ZO-1- or E-Cadherin-positive scores and disorganization scores of E-Cadherin-positive villous epithelium between PEDV-infected and uninfected nursing pigs, or between different time-points in the same group, were analyzed and compared by a Student's *t*-test using GraphPad Prism software (GraphPad Prism Inc.). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical observations in PEDV-inoculated nursing piglets

Clinical signs were first detected at PID 1 in PEDV-inoculated nursing piglets. All inoculated nursing piglets at PIDs 1–5 exhibited acute, severe watery diarrhea and/or vomiting, followed by lethargy and dehydration. No negative control nursing pigs showed diarrhea or other clinical signs throughout the experiment.

3.2. Gross and histologic lesions and immunohistochemistry for the detection of PEDV antigen in PEDV-inoculated nursing piglets

By macroscopic examination, all inoculated nursing pigs tested at PIDs 1–5 exhibited extensively thin and transparent intestinal walls and accumulation of large amounts of yellowish fluid in the small and large intestinal lumen. The other internal organs appeared normal. In inoculated nursing pigs, histologic lesions were limited to the small intestine, mainly jejunum and ileum, and included acute diffuse, severe atrophic enteritis. No histologic lesions were evident in the large intestine or other organs of the inoculated nursing pigs and negative controls. Mean (\pm SDM) jejunal VH:CD ratios of uninoculated

nursing piglets were 8.7 (± 1.4) at PID 1, 7.6 (± 1.2) at PID 3, and 7.1 (± 1.1) at PID 5. On the other hand, mean (\pm SDM) jejunal VH:CD ratios of inoculated nursing piglets were 1.4 (± 0.5) at PID 1, 1.2 (± 0.5) at PID 3, and 1.1 (± 0.6) at PID 5. All inoculated pigs exhibited PEDV antigen-positive cells in the small and large intestine tested at PIDs 1–5. Under our IHC conditions, mean (\pm SDM) PEDV antigen-positive cells per jejunal villus of inoculated nursing pigs were 9.3 (± 7.3) at PID 1, 13.9 (± 5.9) at PID 3, and 9.2 (± 4.3) at PID 5. More detailed clinical disease, pathology, fecal shedding, and viremia (viral RNA in serum) results are in a manuscript in preparation by Jung et al., 2015 (Unpublished data). No other internal organs of the inoculated pigs showed IHC-positive staining. No PEDV antigen-positive cells were detected in the negative control pigs.

3.3. ZO-1-positive epithelial cells in the small and large intestine of PEDV-inoculated nursing piglets

In frozen jejunal (or ileal) and colonic (or cecal) tissues of the uninfected nursing pigs at PIDs 1–5, the major amount of ZO-1 protein were regularly and discontinuously expressed on the apical surface of villous and crypt epithelial cells by IF staining (Fig. 1A–C). Transversely sectioned cells frequently exhibited rectangular, pentagonal, or hexagonal IF staining for ZO-1 (Fig. 1A–F). In the jejunum and colon of all uninfected nursing pigs tested at PIDs 1–5, the entire villous (ZO-1-positive scores, all 3) or crypt (ZO-1-positive scores, all 3) epithelium was mostly positive for ZO-1 protein (Fig. 1A–C, G and H). No differences in staining results and patterns were evident between jejunal and ileal tissues, or between cecal and colonic tissues.

On the other hand, PEDV-infected pigs at PIDs 1–5 had moderately to extensively disorganized, irregular distribution and reduced expression of ZO-1 in villous epithelial cells of the small intestine, but not crypt epithelial cells (Fig. 1D and E), compared to the negative controls (Fig. 1A and B). Regardless of the infection stage, most of the crypt epithelium had mildly reduced expression of ZO-1 protein or numbers of ZO-1-positive cells (mean ZO-1 positive scores: 2.7 at PID 1, 2.7 at PID 3, and 2.8 at PID 5) (Fig. 1D, E, and H), compared to the negative controls.

PEDV-inoculated nursing pigs exhibited small numbers of ZO-1-positive cells at PIDs 1 and 3 (mean ZO-1-positive scores, all 1.2) (Fig. 1D and G) and moderate numbers of ZO-1-positive cells at PID 5 (mean ZO-1-positive score, 2.2) (Fig. 1E and G) in the villous epithelium of the small intestine. Mean ZO-1-positive scores at PID 5 were significantly higher than those at PID 1 or PID 3. At PIDs 1–5, mean ZO-1-positive scores in the jejunal villous epithelium of infected nursing pigs were significantly lower than those in the uninfected pigs ($P < 0.01$ at PIDs 1 and 3; and $P < 0.05$ at PID 5). However, there were no significant differences in mean ZO-1-positive scores in the colonic villous and crypt epithelium between uninfected and PEDV-infected nursing pigs at PIDs 1–5 (Fig. 1C and F).

3.4. E-Cadherin-positive epithelial cells in the small and large intestine of PEDV-inoculated nursing piglets

Apart from ZO-1 that was detected mainly on the apical surface of intestinal epithelial cells under the conditions

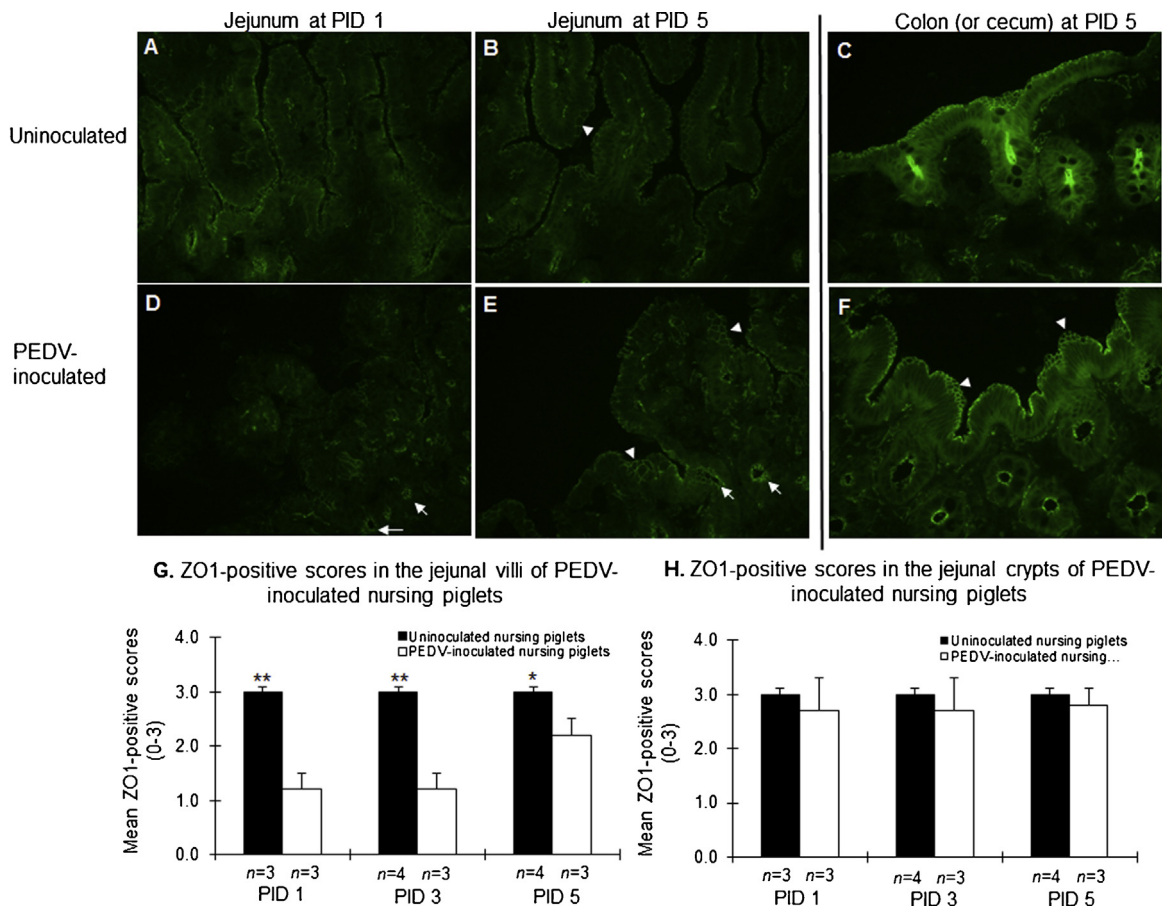
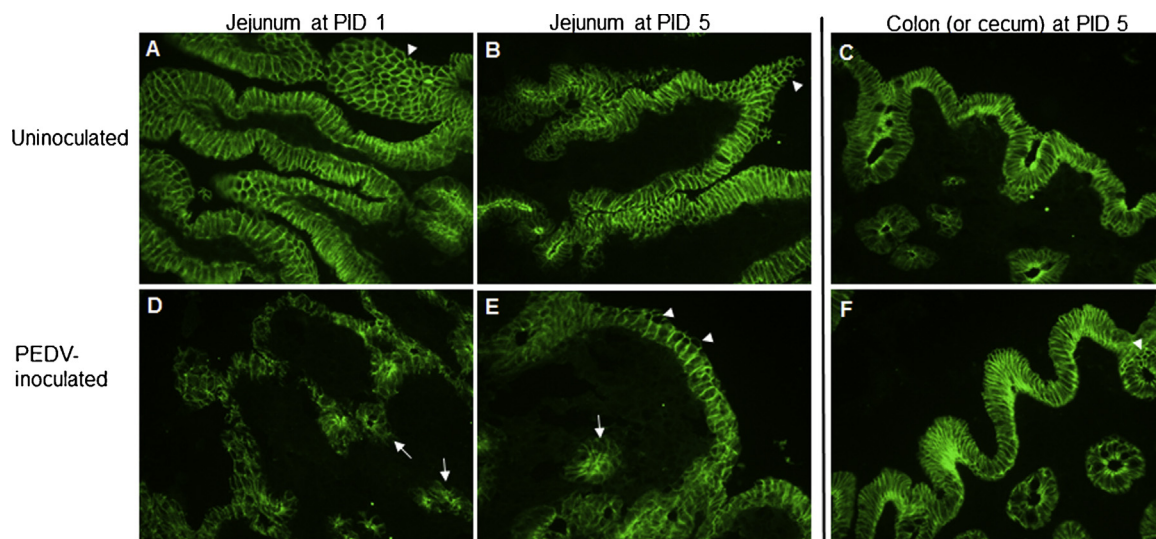


Fig. 1. Detection of the tight junction protein, zonula occludin (ZO)-1, by immunofluorescence (IF) staining. (A and B) Jejunum of uninfected nursing pig at PID 1 (A) and PID 5 (B), showing well-organized distribution and extensive expression of ZO-1 (arrowhead) on the apical surface of villous and crypt epithelial cells. (C) Colon of uninfected nursing pig at PID 5, showing well-organized distribution and extensive expression of ZO-1 on the apical surface of villous and crypt epithelial cells. (D) Jejunum of PEDV-infected pig at PID 1, showing extensively disorganized, irregular distribution and reduced expression of ZO-1 on the apical surface of villous epithelial cells. (E) Jejunum of PEDV-infected pig at PID 5, showing moderately disorganized, irregular distribution and reduced expression of ZO-1 on the apical surface of villous epithelial cells. Note extensive expression of ZO-1 on the apical surface of crypt epithelial cells (arrows) (D and E), and well-organized distribution of ZO-1 in several areas of villous epithelium (arrowheads) (E). (F) Colon of PEDV-infected nursing pig at PID 5, showing well-organized distribution and extensive expression of ZO-1 (arrowheads) on the apical surface of villous and crypt epithelial cells. Note that transversely sectioned cells show rectangular, pentagonal, or hexagonal IF staining for ZO-1 (arrowheads) (B, E, and F). Original magnification, all $\times 400$. Mean ZO-1-positive scores in the jejunal villous (G) or crypt epithelium (H) of PEDV-infected nursing piglets compared to uninfected pigs. ZO-1-positive scores were computed by estimating the number of IF-positive cells in the intestinal section per microscopic area, at $\times 400$ magnification based on the following criteria: 0, no positive cells; 1, 1–29% of ZO-1-positive villous or crypt epithelium showed staining; 2, 30–69% of ZO-1-positive villous or crypt epithelium showed staining; and 3, 70–100% of ZO-1-positive villous or crypt epithelium showed staining. Each bar represents the mean \pm SDM. ** $P < 0.01$; * $P < 0.05$ (statistically significant differences between the PEDV-infected and uninfected nursing pigs by Student's *t*-test).

tested, the large amount of E-Cadherin protein were strongly expressed on the apical and basolateral surface and also mildly in the cytoplasm of the negative control pigs (Fig. 2A and C). Similar to ZO-1 staining, the transversely sectioned cells frequently exhibited rectangular, pentagonal, or hexagonal IF staining for E-Cadherin (Fig. 2A–F). In the jejunum (or ileum) and colon (or cecum) of all uninfected and PEDV-infected nursing pigs tested at PIDs 1–5, the entire villous (E-Cadherin-positive scores, all 3) and crypt (E-Cadherin-positive scores, all 3) epithelium was positive for E-Cadherin (Fig. 1A–F). As a result, there were no differences in mean E-Cadherin-positive scores between uninfected and PEDV-infected pigs. Also, no differences in staining results and patterns were detected

between jejunal and ileal tissues, or between cecal and colonic tissues.

PEDV-infected pigs at PIDs 1–5 showed disorganized, irregular distribution and mildly to moderately reduced expression of E-Cadherin in only villous epithelial cells of the small intestine (Fig. 2D and E), compared to the negative controls (Fig. 2A and B). E-Cadherin-stained tissues were additionally scored based on the extent of disorganization of E-Cadherin-positive villous epithelium. Mean disorganization scores (\pm SDM) of E-Cadherin-positive villous epithelium of the infected pigs were 2.3 (\pm 0.8) at PID 1 and 1.8 (\pm 0.3) at PID 3, which were significantly higher than those (mean disorganization scores, all 0) in the uninfected pigs at the same time-point ($P < 0.01$ at each



G. Disorganization scores of E-Cadherin-positive villous epithelium in jejunum of PEDV-inoculated nursing piglets

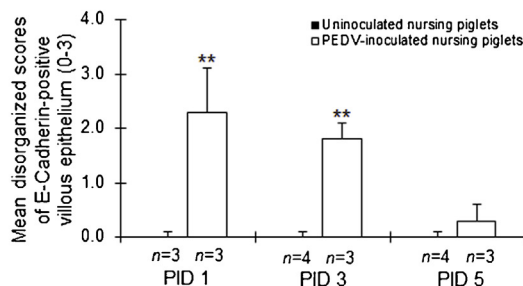


Fig. 2. Detection of the adherens junction protein, E-Cadherin, by immunofluorescence (IF) staining. (A and B) Jejunum of uninfected nursing pig at PID 1 (A) and PID 5 (B), showing well-organized distribution and extensive expression of E-Cadherin (arrowheads) on the apical and basolateral surface of villous epithelial cells and also mildly in the cytoplasm. (C) Colon of uninfected nursing pig at PID 5, showing well-organized distribution and extensive expression of E-Cadherin on the apical surface of villous and crypt epithelial cells and also mildly in the cytoplasm. (D) Jejunum of PEDV-infected pig at PID 1, showing extensively disorganized, irregular distribution and moderately decreased expression of E-Cadherin on the apical and basolateral surface of villous epithelial cells. (E) Jejunum of PEDV-infected pig at PID 5, showing mildly disorganized, irregular distribution and reduced expression of E-Cadherin on the apical surface of villous epithelial cells. Note extensive expression of E-Cadherin on the apical surface of crypt epithelial cells (arrows) (D and E), and well-organized distribution E-Cadherin on the villous epithelium (arrowheads) (E). (F) Colon of PEDV-infected nursing pig at PID 5, showing well-organized distribution and extensive expression of E-Cadherin (arrowhead) on the apical surface of villous and crypt epithelial cells and also mildly in the cytoplasm. Note that transversely sectioned cells show rectangular, pentagonal, or hexagonal IF staining for E-Cadherin (arrowheads) (A, B, E, and F). Original magnification, all $\times 400$. (G) Mean disorganization scores of E-Cadherin-positive jejunal villous epithelium of PEDV-infected nursing piglets compared to uninfected pigs. E-Cadherin-stained tissues were scored by the following system: 0, no positive cells; 1, 1–29% of E-Cadherin-positive villous epithelium showed disorganization; 2, 30–69% of E-Cadherin-positive villous epithelium showed disorganization; and 3, 70–100% of E-Cadherin-positive villous epithelium showed disorganization. Each bar represents the mean \pm SDM. ** $P < 0.01$ (statistically significant differences between the PEDV-infected and uninfected nursing pigs by Student's *t*-test).

time-point) (Fig. 2G). At PID 5, however, there were no significant differences in mean disorganization scores of E-Cadherin-positive villous epithelium between uninfected and PEDV-infected nursing pigs (Fig. 2G).

4. Discussion

Based on our data, PEDV infection resulted in structurally altered TJ and AJ in the villous (not crypt) epithelium of the primary sites of infection, jejunum and ileum, but not the large intestine (cecum and colon). Similar to PEDV infection of intestinal epithelium, TJ and AJ disruption in pulmonary epithelium, which might contribute to the desquamation of the alveolar wall, was observed in lung

biopsies from the *Betacoronavirus* SARS coronavirus (SARS-CoV)-infected macaques and patients (Nicholls et al., 2003). A previous study suggested that alteration of TJ and AJ may create a breach in the epithelial barrier allowing SARS-CoV to reach the basal matrix and eventually the systemic circulation, i.e. viremia (Teoh et al., 2010). By similar mechanisms, after PEDV infection, viremia might be caused during acute infection stages, as reported previously (Jung et al., 2014). In this study, by qRT-PCR, all inoculated nursing pigs tested at PIDs 1–5 also had low to moderate viral RNA titers in serum, ranging from 5.6 to 8.6 \log_{10} GE/ml. Another study showed that impaired TJ in intestinal epithelium of patients with human immunodeficiency virus likely contributes to intestinal barrier dysfunction, resulting in

increased permeability and microbial translocation that cause severe, chronic diarrhea (Chung et al., 2014). The impaired TJ and AJ caused by PEDV might be associated with high co-infection rates of infected nursing piglets with other enteropathogens, such as *Escherichia coli* (Turgeon et al., 1980; Wang et al., 2013).

An *in vitro* study using the porcine intestinal epithelial cell line, IPEC-J2, reported a significantly decreased expression (by western blotting) of TJ and AJ proteins at 40–60 mins after inoculation with another *Alphacoronavirus*, transmissible gastroenteritis virus, or along with PEDV, but not PEDV alone (Zhao et al., 2014). Whether PEDV can alter TJ or AJ *in vivo* in the intestinal epithelium of PEDV-infected pigs was uncertain. Our study clearly demonstrated that at PIDs 1–5, PEDV-infected pigs exhibited mildly to extensively disorganized, irregular distribution and reduced expression of ZO-1 or E-Cadherin in villous, but not crypt epithelial cells of the jejunum and ileum, but not in the large intestine, when compared to the negative controls. Structural alterations of TJ and AJ were extensive in the small intestine of PEDV-infected pigs at PIDs 1–3, but then appeared to recover at PID 5, as evident by increased numbers of ZO-1-positive epithelial cells and markedly improved appearance of E-Cadherin-positive villous epithelium of the infected pigs. Structural destructions and disorganizations of TJ and AJ in the intestinal villous epithelium with PEDV infection might be reversibly recovered during the late stage of infection.

5. Conclusion

Our present study revealed evidence of a possible involvement of structurally impaired TJ and AJ and reduced expression of the related proteins (ZO-1 and E-Cadherin) in the pathogenesis of PEDV. The PEDV-infected intestine may have an impaired gut integrity, possibly leading to uptake of luminal bacteria and loss of water into the intestinal lumen with high osmotic pressure caused by PEDV infection; however, further studies are needed to delineate the functional mechanisms involved.

Conflict of interest statement

Neither of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

- Chung, C.Y., Alden, S.L., Funderburg, N.T., Fu, P., Levine, A.D., 2014. Progressive proximal-to-distal reduction in expression of the tight junction complex in colonic epithelium of virally-suppressed HIV+ individuals. *PLoS Pathog.* 10, e1004198.
- Hartsock, A., Nelson, W.J., 2008. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim. Biophys. Acta* 1778, 660–669.
- Jung, K., Kim, J., Ha, Y., Choi, C., Chae, C., 2006. The effects of transplacental porcine circovirus type 2 infection on porcine epidemic diarrhoea virus-induced enteritis in preweaning piglets. *Vet. J.* 171, 445–450.
- Jung, K., Renukaradhya, G.J., Alekseev, K.P., Fang, Y., Tang, Y., Saif, L.J., 2009. Porcine reproductive and respiratory syndrome virus modifies innate immunity and alters disease outcome in pigs subsequently infected with porcine respiratory coronavirus: implications for respiratory viral co-infections. *J. Gen. Virol.* 90, 2713–2723.
- Jung, K., Saif, L.J., 2015. Porcine epidemic diarrhoea virus infection: etiology, epidemiology, pathogenesis and immunoprophylaxis. *Vet. J.* <http://dx.doi.org/10.1016/j.tvjl.2015.02.017> (in press).
- Jung, K., Wang, Q., Scheuer, K.A., Lu, Z., Zhang, Y., Saif, L.J., 2014. Pathology of US porcine epidemic diarrhoea virus strain PC21A in gnotobiotic pigs. *Emerg. Infect. Dis.* 20, 662–665.
- Nicholls, J., Dong, X.P., Jiang, G., Peiris, M., 2003. SARS: clinical virology and pathogenesis. *Respirology* 8 (Suppl.), S6–S8.
- Saif, L.J., Pensaert, M.P., Sestak, K., Yeo, S.G., Jung, K., 2012. Coronaviruses. In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), *Diseases of Swine*. Wiley-Blackwell, Iowa State University, pp. 501–524.
- Su, C.W., Cao, Y., Kaplan, J., Zhang, M., Li, W., Conroy, M., Walker, W.A., Shi, H.N., 2011. Duodenal helminth infection alters barrier function of the colonic epithelium via adaptive immune activation. *Infect. Immun.* 79, 2285–2294.
- Teoh, K.T., Siu, Y.L., Chan, W.L., Schluter, M.A., Liu, C.J., Peiris, J.S., Bruzzone, R., Margolis, B., Nal, B., 2010. The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Mol. Biol. Cell* 21, 3838–3852.
- Turgeon, D.C., Morin, M., Jolette, J., Higgins, R., Marsolais, G., DiFranco, E., 1980. Coronavirus-like particles associated with diarrhoea in baby pigs in Quebec. *Can. Vet. J.* 21, 100–110.
- Wang, J., Zhao, P., Guo, L., Liu, Y., Du, Y., Ren, S., Li, J., Zhang, Y., Fan, Y., Huang, B., Liu, S., Wu, J., 2013. Porcine epidemic diarrhoea virus variants with high pathogenicity, China. *Emerg. Infect. Dis.* 19, 2048–2049.
- Zhao, S., Gao, J., Zhu, L., Yang, Q., 2014. Transmissible gastroenteritis virus and porcine epidemic diarrhoea virus infection induces dramatic changes in the tight junctions and microfilaments of polarized IPEC-J2 cells. *Virus Res.* 192, 34–45.