Vaccination of sows with a dendritic cell-targeted porcine epidemic diarrhea virus S1 protein-based candidate vaccine reduced viral shedding but exacerbated gross pathological lesions in suckling neonatal piglets

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Abstract
Porcine epidemic diarrhea virus (PEDV) poses a serious threat to swine worldwide as evidenced by its recent introduction into the USA and the devastating economic impact it caused to the USA swine industry. Commercial vaccines against PEDV are available but their efficacies are inadequate. Therefore, vaccines with improved efficacy are needed to effectively control PEDV infections. We previously determined the immunogenicity of a novel dendritic cell (DC)-targeted PEDV S1 protein-based subunit vaccine in weaned piglets in which the PEDV antigen was targeted to DCs through a porcine Langerin-specific antibody. In this study, we evaluated the protective efficacy of this DC-targeting vaccine by immunizing sows at 5 and 2 weeks prior to farrowing and by challenging the 5-day-old piglets with PEDV. The results showed that immunization of sow with DC-targeted PEDV vaccine did not eliminate faecal virus shedding in piglets but significantly reduced faecal viral RNA levels in the early days after virus challenge. The vaccine also reduced the amount of PEDV antigen in intestinal tissues presented with intestinal villi regrowth. However, the DC-targeted vaccine neither mitigated PEDV clinical signs nor affected viral RNA loads in intestinal tissues of piglets. In the vaccinated sow, DC-targeted PEDV vaccine enhanced T helper 1-like cluster of differentiation (CD)4 T cell responses and induced IgG but not IgA-specific immune responses. The suckling piglets in the DC-targeted vaccine group showed increased gross pathological lesions in the small intestine. Results in this study provide insights into the effects of sow cellular immune responses to PEDV infection in suckling piglets.

INTRODUCTION
Porcine epidemic diarrhea virus (PEDV) is endemic causing immense economic losses in pig-raising countries in Asia such as China, Japan and Korea [1]. In the USA, PEDV emerged in 2013 [2] and caused devastating effects on the USA swine industry during the first couple of years of the outbreaks [3]. Currently, PEDV clinical cases in the USA have been reduced to low levels mainly due to strict biosecurity measures implemented in swine farms (www.aphis.usda.gov/animal-health/secd). In Europe, sporadic cases of clinical PED are observed in certain countries such as Germany [4], but are absent in other countries such as Denmark [5]. Modified live-attenuated vaccines (MLVs) have been used for PEDV worldwide, however their efficacies are questionable because of inconsistencies observed in establishing solid lactogenic immunity in vaccinated sows [6].

In the USA, two conditionally licensed vaccines are available on the market at present: an alphavirus vector-based vaccine (Harris Vaccine) and a killed virus PEDV vaccine (Zoetis). However, detailed analyses of the efficacies of these vaccines have not been carried out even though limited studies showed that they are inefficient [7, 8]. In Asia, MLVs have been in use in sow farms with PEDV outbreaks [6]. Even though these MLVs reduce neonatal piglet mortality, they neither prevent faecal virus shedding nor reduce the clinical signs of PEDV in neonatal piglets [6]. On the other hand, the inactivated PEDV vaccine has increased the protective
effect after booster vaccination in gilts or sows previously exposed to live PEDV [9]. PEDV S1 protein is the major determinant of virus neutralizing activity [10–12], and S1 protein-based recombinant vaccine has been reported to induce protective immunity against virus challenge in piglets [13, 14].

We previously reported a novel PEDV S1 protein-based dendritic cell (DC)-targeted vaccine which is highly immunogenic in weaned piglets [15]. In that study, the antigen was directly targeted to porcine DCs through an anti-Langerin antibody to improve the efficiency of antigen presentation to T cells. The study demonstrated that porcine Langerin-targeting significantly improved cellular immune responses against the targeted antigen while showing no influence on the humoral responses when cholera toxin was co-administered as adjuvant [15]. In the present study, we evaluated the protective efficacy of the Langerin-targeted PEDV S1 protein-based candidate vaccine by sow vaccination and virus challenge in piglets.

RESULTS

Antibody responses in vaccinated sows

PEDV S1-specific antibody levels were measured in serum and colostrum samples of sows by ELISA. PEDV S1-specific IgG antibodies were detectable in serum of sow (ID 1978) immunized with DC-targeted PEDV vaccine in a week after boosting [28 days post-first vaccination (DPV)] (Fig. 1a) and also in colostrum collected within 24 h after farrowing (Fig. 1c), however there was no IgA response detected at any time point in serum (Fig. 1b). In contrast, sow (ID 1984) immunized with the killed PEDV commercial vaccine showed strong IgG (both in serum and colostrum) and serum IgA responses while the non-immunized sow (ID 112) in the challenge control group showed no antibody response (Fig. 1a–c). The sow 1978 showed serum neutralizing antibody levels below the detection limit [fluorescent focus neutralization (FFN) titre: less than 1:20] at every time point after vaccination, and showed a barely detectable level of neutralizing antibodies in the colostrum sample (FFN titre: 1:20) but not in milk samples (Table 1). In contrast, in sow 1984, the neutralizing antibody levels were detected at strong levels in serum however only after booster vaccination (FFN titre: 1:160), in colostrum (FFN titre: 1:160) and in milk samples (FFN titres: 1:20–1:80) (Table 1). Sow 112 showed no evidence of neutralizing antibodies in serum, colostrum or milk samples (FFN titre: less than 1:20). In summary, DC-targeted PEDV subunit vaccine induced barely detectable amounts of PEDV neutralizing antibodies in the vaccinated sow.

Cellular immune responses in vaccinated sows

PBMCs were stimulated in vitro with PEDV S1 antigen, stained for intracellular cytokines and analysed by flow cytometry. Sow 1978 showed peak IFN-γ-specific T cell frequencies at 7 DPV in cluster of differentiation (CD)4 T cells (frequency: 0.008) as compared to that in the control group (frequency: 0.000) (Fig. 2a). The PEDV S1-specific CD4 T cells in sow 1978 were predominantly CD4posCD8neg T and CD4negCD8pos T cells (Fig. 2b, c). In sow 1978, the IFN-γ-specific responses were detectable in the CD4posCD8pos T cell compartment until 28 DPV (frequency: 0.004) as compared with that of the control group (frequency: 0.001). On the other hand, in sow 1984, IFN-γ-specific responses were transient with peak IFN-γ-specific CD4 T cell frequencies detected at 14 DPV (Fig. 2a–c).

PEDV S1-specific IL-4 responses were detected only at 7 DPV in sow 1978 in the CD4posCD8pos T cell compartment (frequency: 0.005) as compared with that of the control group (frequency: 0.001) (Fig. 2d, e). However, PEDV S1-
specific IL-4 responses were detected transiently in sow 1984 only after booster vaccination (28 DPV) in the CD4\textsuperscript{pos}CD8\textsuperscript{pos} T cell compartment (frequency: 0.003) as compared with that of the control group (frequency: 0.000) (Fig. 2e). In summary, the DC-targeted PEDV vaccine induced strong and persisting levels of IFN-γ-specific T cell responses in the vaccinated sow.

**Faecal viral shedding in piglets after virus challenge**

Piglets in all treatment groups exhibited clinical signs with no significant treatment differences and most of them survived until the end of the experiment (Fig. 3a–c). All virus-challenged piglets shed the virus in faeces until survival or the end of the study (Fig. 4). Faecal viral RNA levels as measured by quantitative reverse transcription PCR (RT-qPCR) were significantly reduced in the 1978 and 1984 sow groups as compared with that of the control group on 1 and 3 days post-challenge (DPC) (one-way ANOVA, Holm–Sidak’s test, \(P \leq 0.05\)) (Fig. 4a). However, there were no significant differences observed in faecal viral RNA levels between piglets of all treatment groups on 2, 4 and 5 DPC (Fig. 4a). PEDV viral loads in the intestinal tissues (duodenum, jejunum and ileum) and mesenteric lymph nodes of

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<th>Sow treatment group</th>
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<td></td>
<td>0DPV</td>
<td>7DPV</td>
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<td>Challenge control</td>
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<td>3B3scFvFc-PEDVAg</td>
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*The amount of PEDV neutralizing antibodies in the serum as well as in colostrum/milk samples was measured by FFN assay performed in Vero cells using the PEDV strain USA/Colorado/2013. The colostrum/milk samples were defattened before analysis in the FFN assay. The detection limit of the FFN assay is 1:20.

DPV, days post-first vaccination; DPF, days post-farrowing.

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**Table 1. PEDV neutralizing antibody levels in serum and colostrum/milk samples of sows**

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**Fig. 2.** Langerin-targeted PEDV S1 antigen induced IFN-γ-specific T cell responses in both CD4 and CD8 T cell compartments. Peripheral blood mononuclear cells (PBMCs) were stimulated in vitro with recombinant PEDV S1 antigen, stained for CD3, CD4, CD8a surface markers followed by indicated intracellular cytokines, and analysed by flow cytometry. IFN-γ-specific T cell responses were analysed in the total CD4 T cell population (a) and indicated T cell compartments (b, c) in sows of different treatment groups. IL-4-specific T cell responses were shown in the total CD4 T cell population (d) and CD4CD8 double positive T cells (e). Data in the y-axis indicate the T cell frequencies expressed as parental population percentages. Dashed line with \(\bullet\) represents the challenge control, solid line with \(\blacksquare\) represents the experimental DC-targeting vaccine group, and solid line with \(\blacktriangle\) represents the commercial PEDV vaccine group.
piglets were not significantly different between treatment groups (Fig. 4b–e). Altogether, the vaccination of sow with langerin-targeted PEDV vaccine did not completely prevent PEDV faecal shedding but reduced the viral RNA levels in faeces of virus-challenged piglets.

Gross pathological lesions in virus-challenged piglets

Gross pathological lesions due to PEDV infection and the nature of intestinal content were assessed in piglets by a board-certified veterinary pathologist (TL) who was blinded to treatment status. The gross lesion scores were adjusted to the percentage of affected tissue showing a particular gross lesion score. Most gross lesions were observed in the small intestine followed by the cecum and colon (Fig. 5). When compared to the control and sow 1984 groups, piglets in the sow 1978 group had significantly increased gross lesions in the small intestine and cecum (one-way ANOVA, Holm–Sidak’s test, $P < 0.05$) but not in the colon (Fig. 5a–c). The mean content score of piglets in the sow 1978 group was significantly higher in the small intestine but not in the cecum and colon as compared with that of the control group (one-way ANOVA, Holm–Sidak’s test, $P < 0.0001$) (Fig. 5d–f). Overall, the sow immune responses induced by DC-targeted PEDV vaccine increased gross intestinal lesions associated with watery content in suckling piglets challenged with PEDV.

Histopathological lesions in virus-challenged piglets

Microscopic lesions due to PEDV were analysed in challenged piglets at necropsy by a veterinary pathologist who was blinded to treatment status. PEDV antigen [immunohistochemistry (IHC) signal] intensity scoring on formalin-fixed intestinal tissues revealed that piglets in the sow 1978 and 1984 groups showed significantly reduced PEDV antigen levels in the duodenum and jejunum but not in the ileum and colon as compared to that of the control group (one-way ANOVA, Holm–Sidak’s test, $P < 0.0001$) (Fig. 6a–d). Similarly, the mean ratios of villous length and crypt depth in jejunum tissues of piglets in the sow 1978 and 1984 groups were significantly increased as compared to that in the control group (one-way ANOVA, Holm–Sidak’s test, $P < 0.0001$) (Fig. 7). In summary, the immunization of sow with DC-targeted PEDV antigen significantly reduced the amount of PEDV antigen in the intestines of suckling piglets with evidence of decreased intestinal villous damage.

DISCUSSION

Specific targeting of PEDV S1 antigen to DCs augmented the CD4 T cell-specific immune responses in weaned piglets without affecting the development of antibody responses [15]. In this present study, we examined whether enhanced cellular immune responses against DC-targeted PEDV vaccination in a sow vaccination model translated into better protection in suckling piglets challenged with the virus. Even though DC-targeted PEDV S1 antigen induced barely detectable PEDV neutralizing antibodies in colostrum but not in milk, there was a significant reduction in virus shedding in faeces during the early time points (1 and 3 DPC) after virus challenge. At necropsy (5 DPC), there was a great reduction in the amount of PEDV antigen in intestinal tissues of piglets in the DC-targeted vaccine group and significant intestinal villous protection compared to the control pigs. Surprisingly, the lactation immunity conferred by DC-targeted PEDV S1 vaccine actually increased the gross intestinal lesions in virus-challenged piglets. In this study, the virus-challenged piglets are derived from the same vaccinated sow, and therefore the effects of genetic variation on
protection efficacy of DC-targeted PEDV S1 vaccine are not accounted in this study.

Langerin-targeting of PEDV S1 subunits induced strong PEDV neutralizing antibodies in the serum of weaned piglets [15]. However, in the present study the Langerin-targeted vaccine antigen induced no or lower neutralizing antibody responses in sow serum and colostrum. This observation may be explained by the differences in vaccine antigen expression systems as well as the adjuvants. There were N-glycosylation differences noticed between baculovirus- and mammalian-expressed PEDV S1 antigens [14] owing to the lack of complex glycosylations in insect cells [16]. Therefore, not all neutralizing epitopes in baculovirus-expressed PEDV S1 protein are expected to be in the proper configuration for effective presentation to B cells, if the conformations of discontinuous epitopes are N-glycosylation-dependent [17]. Nevertheless, in this study, we selected a baculovirus expression platform for preparing the vaccine antigen and a commercially suitable adjuvant for formulating the experimental vaccine, considering the commercial feasibility of the potential vaccine candidate which is intended for sows and gilts.

The levels of neutralizing antibodies in colostrum/milk of vaccinated sows (both Langerin-targeted and killed commercial vaccine groups) correlated with a reduction in the levels of faecal virus shedding in corresponding piglets. The absence of a strong reduction in faecal virus shedding and unaffected tissue viral loads in both vaccine groups may be due to the lack of persistent levels of neutralizing antibodies in colostrum/milk which is a critical factor for PEDV protective immunity in suckling piglets [7, 8]. The higher tissue viral loads in both vaccine groups indicate that the challenge virus replication in the intestine of piglets was unaffected by vaccine-induced lactogenic immune responses in both vaccine groups which are further confirmed by the observation of similar peak viral RNA shedding in faeces (2 DPC). In this study, we intentionally challenged piglets with a non-lethal PEDV dose to assess the protective effects of the
vaccine on the main clinical presentations of PEDV: reduction in diarrhea/vomiting as well as on faecal viral shedding. Unfortunately, the experimental vaccine or the commercial PEDV vaccine did not reduce the clinical presentations of PEDV even at a low non-lethal PEDV dose. Therefore, it is expected that the Langerin-targeted vaccine would not have reduced the clinical presentations of PEDV at a higher challenge dose including a lethal PEDV dose.

Interestingly, since the IgG2 isotype is the predominant isotype produced in immune responses biased toward Th1 cellular immunity against viruses [18], it is highly possible that the vaccine-specific antibodies detected in the sow serum and colostrum in the Langerin-targeted vaccine group are predominantly IgG2 antibodies. Virus-specific IgG2 antibodies showed low virus neutralizing activities in vitro but they efficiently cleared virus in vivo with enhanced virus neutralization capabilities as well as through antibody-dependent cell cytotoxicity [19–21]. In the sow vaccinated with Langerin-targeted vaccine, in the absence of significant IgA responses, IgG antibodies (including IgG2 isotype) are expected to be secreted in the colostrum which, after systemic absorption [22], might have contributed partly to viral clearance [23]. This is corroborated by the observation that the number of virus-producing cells as measured by PEDV viral antigen (IHC signal) intensity in intestinal tissues was significantly reduced in the Langerin-targeted vaccine group with a significant improvement in villous growth at day 5 after challenge.

Exacerbated gross lesions in piglet intestines in the Langerin-targeted vaccine group might have been due to immunopathogenesis as a result of unwanted IgG-mediated complement-activated inflammation [19, 24]. In addition to IgG-mediated responses, T lymphocytes derived through colostrum or milk might also have contributed to antiviral immune responses and increased gross lesions in piglets in the Langerin-targeted vaccine group [25]. As observed in

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Fig. 5. Sow vaccination with Langerin-targeted PEDV S1 subunits increased gross pathological lesions with watery content in the small intestine of virus-challenged piglets. Gross lesions and the nature of intestinal content were scored by a board-certified veterinary pathologist who was blinded to treatment status. Higher lesion scores indicate more severe pathological lesions and higher content scores indicate more watery diarrhea. (a–c) Mean gross pathological lesion scores were calculated for the small intestine (a), cecum (b) and colon (c) of piglets belonging to indicated treatment groups. (d–f) Mean intestinal content scores were indicated for the small intestine (d), cecum (e) and colon (f) of piglets. Data in the y-axis represent mean values ± SEM (n≥8). Significant differences between treatment groups were measured by one-way ANOVA and Holm–Sidak’s multiple comparisons test with α set at 0.05. NS, non-significant (P>0.05), *P≤0.05, **P≤0.01 and ****P≤0.0001.
the previous study [15], the non-targeted S1 vaccine is expected to induce antibody levels similar to that observed with the Langerin-targeted S1 vaccine in the present study. It is interesting to examine whether non-targeted PEDV S1 vaccine induced the same protective phenotype and/or exacerbated gross lesions as Langerin-targeted PEDV S1 vaccine in the sow–piglet model. If the effectors of sow cellular immunity (IgG2 and T cells) are predominantly contributing to the protective phenotype as well as exacerbated gross lesions in piglets of the Langerin-targeted vaccine group, the non-targeted PEDV S1 vaccine is expected not to induce the same protective phenotype with no exacerbation of gross lesions in the sow–piglet model.

Overall, Langerin targeting of the PEDV S1 antigen induced a sustained T cell immune response particularly CD4 T cells showing T helper 1 phenotype which might be associated with IgG2-dominated virus-specific antibody responses. Langerin targeting clearly did not augment IgA responses in the vaccinated sow. The PEDV S1-specific IgG antibodies and possibly

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**Fig. 6.** Passive immunity conferred by Langerin-targeted PEDV S1 subunits reduced PEDV antigen intensity in intestinal tissues of virus-challenged piglets. Formalin-fixed intestinal tissue sections on slides were stained with anti-PEDV NP mAb followed by horse radish peroxidase (HRP)-conjugated secondary reagent and incubation with HRP substrate. The PEDV antigen [immunohistochemistry (IHC) signal] intensities were scored by a board-certified veterinary pathologist who was blinded to treatment status. The PEDV antigen intensities were assessed for different intestinal tissues, duodenum (a), jejunum (b), ileum (c) and colon (d). Data in the y-axis represent mean values ± SEM (n≥8). Significant differences between treatment groups were measured by one-way ANOVA and Holm–Sidak’s multiple comparisons test with α set at 0.05. NS, non-significant (P>0.05), *P≤0.05, **P≤0.01 and ***P≤0.001.
T lymphocytes secreted in colostrum/milk contributed to a moderate reduction in faecal virus shedding at early time points. Moreover, in the Langerin-targeted vaccine group, the immunity via colostrum/milk likely induced immune-mediated pathogenesis at tissue level while they concurrently reduced viral replication in intestinal tissues of suckling piglets. The absence of significant protective effects of DC-targeting vaccine in this study could be due to a reduced neutralizing antibody quantity and/or quality, or none-to-minor contribution of maternal cellular immunity to lactogenic immunity. The results of this study provide new insights into protection effects of lactation immunity induced by Langerin-targeted PEDV S1 antigen in sow on the virus replication and shedding in suckling piglets.

**METHODS**

**Preparation of the DC-targeting vaccine antigen**

The coding DNA sequence of the PEDV vaccine antigen (3B3scFv-pFc-PEDVsAg, [15]) was codon-optimized, custom-synthesized (GenScript) and subsequently cloned in pVL1392 transfer vector (Invitrogen). The recombinant baculovirus was prepared by co-transfecting pVL1392 vector along with BacPAK6 viral DNA (Clontech) into Sf9 cells using Bacfectin (Clontech). The progeny baculovirus was plaque-purified and high-titre working stocks were prepared in BTI-TN5B1-4 (HighFive) cells. The DC-targeting vaccine antigen was prepared by infecting BTI-TN5B1-4 cells with the recombinant baculovirus (at least 2 m.o.i.), and purified from culture supernatant using Captiva Pri-Mab Protein A beads (Repligen). The molecular size and identity of vaccine antigen was confirmed by SDS-PAGE with Coomassie blue staining and immunoblotting using anti-PEDV S1 mAb (Medgene labs) respectively (Fig. S1, available in the online version of this article).

**Animal study**

Three PEDV-negative pregnant sows at 65 days of gestation were randomly allocated into three treatment groups: challenge control (sow 112), experimental DC-targeting vaccine group (sow 1978) and a commercial PEDV vaccine group (sow 1984) (Table 1). At 5 weeks prior to farrowing, sows 1978 and 1984 were intramuscularly vaccinated with 200 µg 3B3scFv-pFc-PEDVsAg antigen [15] mixed with a commercial proprietary adjuvant or with a killed PEDV commercial vaccine (Zoetis), respectively. At 5 weeks prior to farrowing, the sow 112 received PBS as the non-vaccinated control. At 2 weeks prior to farrowing, all three sows received a second dose of booster vaccines of the same kind, through the same route. Weekly blood samples were collected for measuring antibody and cellular immune responses in sows.

After farrowing, litter sizes of surviving newborn piglets ranged between 8 and 20. Piglets were randomly selected within a treatment group with sample sizes from 8 to 10. At 5 days post-farrowing, piglets were challenged with PEDV USA/Colorado/2013 (National Veterinary Services Laboratories) [26] orally at 10⁵ TCID₅₀/2 ml. Clinical signs were scored daily in piglets between 0–5 days DPC: for vomiting, ‘no vomiting’ (score 0) and ‘sign of vomiting’ (score 1), as well as for diarrhea, faecal scores were calculated as previously described [27–29]. After virus challenge, faecal samples were collected from piglets daily to assess faecal virus shedding by RT-qPCR.

**In vitro antigen stimulation and intracellular cytokine staining**

T cell immune responses in peripheral blood mononuclear cells (PBMCs) were analysed by intracellular cytokine staining and flow cytometry as previously described [30]. Recombinant PEDV S1 antigen was used for stimulating PBMC cultures.

**ELISA and virus neutralization test**

The PEDV S1-specific IgG/IgA antibodies were measured in serum and colostrum samples by ELISA as previously described [15]. Colostrum samples (0.5 ml each) were treated with 15 µl rennet per sample (5 mg ml⁻¹ bovine chymosin, G-Biosciences) at 37 °C for 1 h. Once solidified, the whey was separated by centrifugation at 6000 g for 20 min and the supernatant was collected. Whey samples were...
diluted fourfold with PBS containing 0.05% Tween 20 and 4% non-fat milk, and applied on an ELISA plate.

PEDV neutralizing antibodies were measured by a FFN test in serum, colostrum and milk samples at the Animal Disease Research and Diagnostic Laboratory (ADRDL), South Dakota State University, Brookings, SD. The PEDV USA/Colorado/2013 strain (NVSL) was used as the virus strain in the FFN assay [31].

Quantitative reverse-transcription PCR

Total RNAs were isolated from faecal suspensions (10%) and homogenized tissue samples by Trizol LS reagent (Thermo Fisher Scientific). To prepare a standard curve, in vitro transcribed PEDV template RNAs (201 bases) were prepared using a template plasmid. The standard RNA was prepared by 10-fold serial dilutions. The sequences of primers, Taqman probe, and the thermocycling conditions used in the RT-qPCR were described previously [32], however different quenchers [Internal ZEN and 3‘Iowa Black FQ (IDT technologies)] were incorporated in the probe. The reaction contained (per sample) nuclease-free water (12.25 µl), 400 nM each primer together with 200 nM probe (0.5 µl, IDT technologies), Taqman Fast Virus 1-step master mix (6.25 µl, Applied Biosystems) and sample or standard RNA (6 µl). The PCR reactions for each sample were done in duplicate wells. The standard curve parameters are: PCR efficiency (E)=95%, R²=0.998, slope=−3.448, Y-intercept= 40.814, and the sensitivity of detection limit is '50 RNA copies'.

Gross and histopathological evaluation

Pigs were evaluated for the gross and histopathological lesions immediately after humane end point-determined euthanasia or at the end of the study (5 DPC) by a board-certified veterinary pathologist (TL) who was blinded to treatment status. The intestinal gross lesions, intestinal content and PEDV antigen intensity in intestinal tissue sections were scored as previously described [27]. The villous-length (V) and crypt-depth (C) were calculated by averaging 10 different measurements of villous and crypt per formalin-fixed intestinal tissue sections stained with hematoxylin and eosin. V/C ratio for each section was calculated by dividing the mean villus length with the mean crypt depth. To measure PEDV antigen intensity, the formalin-fixed intestinal tissue sections were deparaffinized by three xylene changes, rehydrated gradually in ethanol-water mix (100–70%) and finally with PBS. The antigen was retrieved with 0.1% Protease E (Sigma) and the endogenous peroxidase was blocked with 3% H₂O₂. Sections were further incubated serially with anti-PEDV NP mouse mAb (Medgene labs, clone SD6-29, 1:200), ImmPRESS anti-mouse Ig (Vector labs) and ImmPRESS DAB peroxidase substrate (Vector labs), which were washed extensively with PBS between incubations. Finally, sections were counterstained with Mayer’s hematoxylin (Sigma), dehydrated and mounted with coverslips.

Statistical analysis

Significant differences between the means of treatment groups were determined by one-way ANOVA and Holm–Sidak’s multiple comparisons test in Graph pad Prism v6. A P-value ≤0.05 was considered as significant.

References


