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Short Communication

Previous infection of sows with a "mild" strain of porcine epidemic diarrhea virus confers protection against infection with a "severe" strain

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ABSTRACT

Porcine epidemic diarrhea virus (PEDv) infected approximately 50% of the US swine breeding herds from July 2013 to July 2014 as estimated by the Swine Health Monitoring Project. In the absence of effective vaccines or standard control protocols, there is an urgent need for evidence of cross-protective immune countermeasures. Here, we evaluated the response of 3-day-old piglets born to sows exposed seven months earlier to a mild strain of PEDv to challenge with a virulent PEDv isolate. Piglet survival to one week of age was 100% compared to 67% in piglets born to sows not previously exposed, and morbidity was 43% compared to 100%, respectively. At necropsy at 7 days of age, the PEDv Ct value was 23.6 (range 16.6–30.6) in intestinal contents, compared to 17.2 (range 15.9–18.5) ($p < 0.06$) in litters from sows with no previous exposure to PEDv. The findings indicated that durable lactogenic immunity was present in sows previously exposed to a mild strain of PEDv and this immunity induced cross-protection to representative virulent PEDv. Thus, a naturally attenuated form of PEDv provided significant passive immune protection for seven months against piglet challenge with virulent PEDv.

1. Introduction

Porcine epidemic diarrhea virus (PEDv) infected approximately 50% of the US swine breeding herds from July 2013 to July 2014 as estimated by the Swine Health Monitoring Project (Goede and Morrison, 2014a,b). PEDv is an enveloped, single-stranded, positive-sense RNA virus of the genus *Alphacoronavirinae* in the family *Coronaviridae* and is related to transmissible gastroenteritis virus (TGEv) (Hofmann and Wyler, 1989; Saif et al., 2012). After whole herd exposure to PEDv via feedback of intestinal and fecal material, breeding herds rapidly develop immunity which reduces the 100% morbidity of watery diarrhea and 50–100% pre-weaning mortality that is seen during an acute outbreak in naïve herds (Saif et al., 2012; Stevenson et al., 2013). Cases of infection with a mild strain of PEDv (OH851) have resulted in reduced clinical impact and faster return to baseline production than typical PEDv (Goede and Morrison, 2014a,b; Vlasova et al., 2014; Wang et al., 2014a). The mild strain is genetically similar to two existing PEDv clades present in US herds but has a deletion in the spike protein (Vlasova et al., 2014; Wang et al., 2014b). Initial observation of drastically different mortality throughout acute outbreak

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and quick resolution of shedding in the environment in some herds experiencing infection with mild PEDv led to increased whole-genome sequencing of viruses in these herds. In addition to less severe clinical disease in sows, piglet loss in litters is 0.4 pigs per sow, versus 2.6 pigs per sow in herds infected with typical, virulent PEDv (Goede and Morrison, 2014a,b).

Little is known about duration of immunity in herds exposed to PEDv and if anecdotal evidence of "re-breaks" in endemically affected populations is due to waning immunity. It is important to determine the cross-protective immune efficacy and duration of immunity of mild strains of PEDv against infection with more severe PEDv isolates.

2. Methods

Two swine herds in the Midwest United States were identified and matched by genetics, management company and health, excluding the history of PEDv infection. Fecal swabs and sera were collected from 30 representative sows in each herd. Fecal swabs were tested by PEDv reverse transcription polymerase chain reaction (RT-PCR) for presence of virus (Kim et al., 2001; Wang et al., 2014b); sera were tested by PEDv enzyme-linked immunosorbent assay (ELISA) (Gerber et al., 2014) for IgG or by immunofluorescence assay (IFA) at the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL). Herd 1 had no previous infection of PEDv based on negative RT-PCR and IFA and no history of clinical signs. Herd 2 was infected with mild PEDv followed by whole herd feedback exposure approximately 7 months prior to the experiment (Geiger and Connor, 2013). The mild PEDv was confirmed by spike protein S1 fragment sequencing (Genbank accession XXX) and was since eliminated from the herd as indicated by 4 negative PEDv PCR rounds of testing on samples of 30 litters submitted in 6 pools and collected at least 1 week apart.

A total of 21 sows (11 from herd 1 and 10 from herd 2) that had already had at least 2 litters and were at a specific gestation day were randomly selected at each farm. Herd 1 sows were randomly selected from a subset of 30 sows that were tested negative by PEDv ELISA. Herd 2 sows were randomly selected from a subset of 30 sows that were tested either positive or negative by PEDv ELISA. Average parity of selected sows was the same between the 2 herds. Six of the 11 sows from herd 1 and 5 sows from the 10 at herd 2 were transported to a BSL-2 animal isolation facility at the University of Minnesota to form experimental groups "A: previously not infected and challenged," and "B: previously infected and challenged." The 5 remaining sows at each herd were experimental groups "C: previously not infected and not challenged," and "D: previously infected and not challenged." One sow from herd 1 was not challenged and served as a negative control sow in the BSL-2 isolation facility throughout the study.

Sows were delivered to the isolation facility at day 108 (±1 day) of gestation and allowed 24 h of acclimation. Challenge-exposure with virulent PEDv was conducted at day 109 of gestation. Sows in groups A and B were challenged with 15 mL of cell culture-derived PEDv (Genbank accession KF267450, Ct = 20, 6% different in S1 nucleic acid sequence from the mild strain) orally (Hofmann and Wyler, 1989; Jung et al., 2014; Madson et al., 2014). Sows that did not farrow by day 115 of gestation were administered 10 mg Lutalyse followed by 40 IU oxytocin 24 h later to induce parturition (Gall and Day, 1987).

Challenge-exposure was performed orally to the 3-day-old piglets with 1 mL of mucosal scrapings containing the same virulent PEDv (Genbank accession KF267450, Ct = 18) via syringe and 2 mL to the sows also orally via syringe (Madson et al., 2014; Jung et al., 2014). Sow feces and piglet feces were collected from each litter on days 1, 4 and 7 of lactation for testing by PEDv RT-PCR. Colostrum and milk samples were collected from each sow on days 0, 3 and 7 of lactation for testing by ELISA for anti-PEDv IgA. Piglet clinical signs were monitored daily and weights were taken at farrowing and 7 days of lactation.

All sows were monitored for clinical signs of anorexia and diarrhea twice daily and fecal samples were collected every other day from the time of sow challenge through day 3 post-farrowing. Clinical signs recorded in piglets included diarrhea, defined as any loose or soft stool observed during defecation or seen on the piglets, dehydration, defined as sunken eyes and observation of wrinkled skin, and vomiting. Mortality, i.e. absence of life, was determined by a veterinarian.

Piglets were euthanized at 7 days post farrowing (dpf) by intravenous injection of pentobarbital into the external jugular vein and were necropsied. Eight sections of jejunum and ileum were collected and immunohistochemical (IHC) staining was performed to evaluate cells for active PEDv infection (Guscetti et al., 1998). Sows were sent to slaughter at 7 dpf. Samples from piglets were evaluated for any indication of passive protection from PEDv clinical signs as defined by either reduced morbidity confirmed by PCR, mortality, or Ct value of intestinal samples at sacrifice. Protection was defined as any of these parameters showing a significant difference between the challenged groups.

Statistical analyses of clinical outcomes of PED including litter morbidity, mortality, and viral shedding in piglet intestines were performed using a non-parametric Wilcoxon rank-sum method with litter as the experimental unit.

3. Results

Sows in groups C and D which remained at their respective breeding herds experienced no signs of clinical infection with PEDv over the duration of the study, and fecal samples from 10 sows and at least 5 piglets per litter through 7 days lactation were negative by PEDv RT-PCR. Clinical signs were not observed and no PEDv genetic material was detected by RT-PCR by day 3 post-farrowing.

One sow from group B (B4 in Table 1) incurred a prolapsed uterus prior to farrowing and was euthanized. The piglet data were not included in the analyses. Another sow prolapsed her uterus following farrowing; the sow was managed with antibiotics and analgesics. Vital signs including temperature, respiration rate, heart rate, mucous membrane color, and capillary refill time were monitored 3 times daily along with feed intake. The sow appeared to have minimal pain and was maintained throughout the