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Pathogenesis of porcine epidemic diarrhea virus isolate (US/Iowa/18984/2013) in 3-week-old weaned pigs

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is associated with clinical diarrhea in naïve swine of all ages. This report describes timing of antibody generation and disease progression following infection with a US PEDV isolate by assessing fecal viral shedding, morphometric analysis of intestinal lesions, and magnitude of immunohistochemical staining. Sixty-three, 3-week-old pigs were randomly allocated into control ($n = 27$) and challenged ($n = 36$) groups. Challenged pigs were administered 1 mL of 1×10^3 PFU/mL of US/Iowa/18984/2013 PEDV isolate by oro-gastric gavage. Three control and four challenged pigs were necropsied on days post-inoculation (dpi) 1, 2, 3, 4, 7, and weekly thereafter, until study termination on dpi 35. Clinical disease, fecal shedding, body weight, and temperature were monitored during the study period. Diarrhea was observed in challenged pigs beginning for some on dpi 2, affecting a majority of pigs by dpi 6 and subsiding by dpi 10. Average daily gain was significantly lower ($P < 0.001$) for one week post-infection in challenged pigs. PEDV was detected in feces by PCR on dpi 1 and continued in a subset of pigs until dpi 24. PEDV-specific antigen was detected in villous enterocytes of challenged pigs by immunohistochemistry (IHC) on dpi 1, 2, 3, 4, 7, and 14. Microscopic lesions included severe diffuse atrophic enteritis with significantly reduced ($P < 0.001$) villous length observed on dpi 3, 4, and 7. Under the conditions of this study, fecal shedding of PEDV and IHC staining can precede and continue beyond the observation of clinical signs, thus increasing the risk of viral transmission.

1. Introduction

Porcine epidemic diarrhea virus (PEDV), an enteric pathogen recently introduced into the United States (US) swine industry, is an enveloped, single-stranded RNA virus and member of the *Coronaviridae* family, genus *Alphacoronavirus* (de Groot et al., 2011). Following fecal-oral transmission, the virus infects and replicates in mature,

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small intestinal enterocytes resulting in villous atrophy that causes a malabsorptive, watery diarrhea with vomiting and anorexia in swine of all ages (Saif et al., 2012). Clinical signs and histopathologic lesions associated with PEDV infection are indistinguishable from those of transmissible gastroenteritis virus (TGEV); however, these alphacoronaviruses are antigenically distinct and do not demonstrate serological cross-reactivity (Pensaert et al., 1981).

Enteric disease associated with PEDV was first recorded in England in the early 1970s and has since spread to other European and Asian countries.(Song and Park, 2012) After the initial introduction and outbreak of PEDV in the United States (April 2013), sequencing of PEDV isolates revealed similar nucleotide homology (>99%) with a Chinese strain entered into GenBank in 2012 (Huang et al., 2013; Stevenson et al., 2013). The source of PEDV introduction into the US swine population has not yet been determined; nonetheless, there have been over 6,000 PEDV-positive accessions to US veterinary diagnostic facilities originating from 30 different states as of May 2014 (http://www.aasv. org).

Because PEDV was a foreign animal disease in the US until 2013, its emergence generated numerous scientific questions from veterinarians, diagnosticians, swine researchers, and the US swine industry. The intent of this study was to characterize the pathogenicity of PEDV isolate US/Iowa/18984/2013 in post-weaned pigs by: 1) assessing clinical disease progression and gross lesions, 2) quantifying fecal viral shedding and antibody production, and 3) analyzing morphometric intestinal lesions and magnitude of immunohistochemical staining.

2. Material and methods

2.1. Animals

Sixty-three, 3-week-old weaned pigs of mixed sex and breed were sourced from a single commercial, cross-bred farrow-to-wean herd with no known prior exposure to PEDV. Pigs were free of porcine reproductive and respiratory syndrome virus and TGEV antibodies. All pigs were given a single dose of a commercially available porcine circovirus type 2 and *Mycoplasma hyopneumoniae* vaccine (Ingelvac® CircoFLEX-MycoFLEX®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) one week prior to arrival, and an intramuscular antibiotic injection (Excede®, Zoetis, Florham Park, NJ), per label instructions, upon arrival at the research facility.

2.2. PEDV inoculum

Porcine epidemic diarrhea virus isolate US/Iowa/18984/2013 (GenBank accession #KF804028) was propagated in Vero cells (ATCC® CCL-81) as previously described (Hofmann and Wyler, 1988). Briefly, this isolate was obtained from a case submission to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) on May 16, 2013 from a 2,500 head farrow-to-wean farm in Northern Iowa experiencing acute onset of diarrhea in suckling pigs. Submitted neonatal intestines demonstrated severe atrophic enteritis and contents were negative for TGEV and Rotavirus serogroups A, B, and C using commercial and in-house PCR assays at the ISU-VDL, respectively. Virus isolation, plaque cloning, and propagation were performed as previously documented (Hoang et al., 2013). The viral inoculum represented a total of six cell-culture passages.

2.3. Study design and housing

Pigs were randomly allocated into control (n= 27) and challenged (n = 36) groups. Groups were housed in the same facility, but separated by room and ventilation system. Pigs in each room were confined by pens on a solid floor that was rinsed daily, fed a balanced diet *ad libitum* based on weight, and given free access to water. PEDV-challenged pigs received a 1 mL dose of 1×10^3 plaque-forming unit (PFU)/mL via oro-gastric gavage using a 12 gauge French catheter flushed with 10 mL of 0.01 M phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO) on days post-inoculation (dpi) 0. Sham-inoculated pigs (controls) were administered volume-matched virus-free cell culture media. Three control and four challenged pigs were randomly selected for necropsy on dpi 1, 2, 3, 4, 7, 14, 21, 28, and 35 (Table 1). The experimental design was approved by the Iowa State University Institutional Animal Care and Use Committee (protocol log #6-13-7593-S).

2.4. Clinical assessment

Body weights were recorded for each pig prior to inoculation and weekly thereafter until study termination. Weekly weights were used for calculating average daily gain (ADG); weekly gain divided by seven. Rectal temperature was recorded for a subset of pigs within each group (nine controls and 12 challenged pigs) that remained alive for the first 7 days post-challenge. In addition, the total number of pigs with clinical diarrhea were subjectively scored for fecal consistency using the following criteria: 1) normal, 2) semi-liquid without a formed consistency (cow-pie feces), and 3) watery/liquid contents. Fecal scores were recorded daily for the first week, dpi 10 and 14, and weekly thereafter.

Table 1
Experimental design for 3-week-old weaned pigs challenged with PEDV isolate US/Iowa/18984/2013.

Group	n	Age	Inoculation[†]	# of pigs necropsied at given day post inoculation								
				1	2	3	4	7	14	21	28	35
Control	27	3 weeks	Sham	3	3	3	3	3	3	3	3	3
Challenged	36	3 weeks	PEDV (1×10^3 PFU/ml)	4	4	4	4	4	4	4	4	4

[†] Inoculation via oro-grastric catheter; 1 mL