Veterinary Microbiology 211 (2017) 58–66

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Effects of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity☆

S.M. Curry[a,*], K.J. Schwartz[b], K.J. Yoon[b], N.K. Gabler[a], E.R. Burrough[b]

[a] Department of Animal Science, Iowa State University, Ames, IA, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA, USA

ARTICLE INFO

Keywords:
Nursery pig
Porcine epidemic diarrhea virus
Sulfomucin
Sialomucin
Barrier function

ABSTRACT

The pig intestinal epithelium can be compromised by pathogens leading to reduced integrity and function. Porcine epidemic diarrhea virus (PEDV), recently detected in North America, exemplifies intestinal epithelial insult. Although several studies have investigated the molecular aspects and host immune response to PEDV, there are little data on the impact of PEDV on pig intestinal physiology. The objective of this study was to investigate the longitudinal impact of PEDV on nursery pig intestinal function and integrity. Fifty recently-weaned, 5-week-old barrows and gilts (BW = 9.92 ± 0.49 kg) were sorted based on body weight (BW) and sex into two treatments: 1) Control or 2) PEDV inoculated. At 2, 5, 7, and 14 days post inoculation (dpi), 4 pigs per treatment were euthanized and jejunum sections collected. PEDV antigen was detected in inoculated pigs by immunohistochemistry in 50% (2/4) at dpi 2, 100% (4/4) at dpi 5, and none at later time points. PEDV-infected pigs had reduced ($P < 0.05$) villus height and decreased transepithelial resistance compared with controls. Total acidic mucins, particularly sialomucin, were reduced in PEDV pigs at dpi 2 and then increased compared with controls at dpi 7 and 14. In addition, PEDV pigs had increased stem cell proliferation ($P < 0.05$) and a numerical increase in DNA fragmentation compared with controls through dpi 7 which coincided with an observed return of digestive function to that of controls. Collectively, these data reveal that PEDV infection results in time-dependent changes not only in intestinal morphology but also barrier integrity and function.

1. Introduction

Porcine epidemic diarrhea virus (PEDV) infects porcine enterocytes (Jung and Saif, 2015) and induces acute mucosal disruption, blunting of affected villi, and thinning of the intestinal walls which result in watery diarrhea, dehydration, and sometimes death (Stevenson et al., 2013). In the first year after its emergence in the United States (Stevenson et al., 2013), PEDV infection was associated with the death of approximately 7 million piglets (Jung and Saif, 2015). While mortality in PEDV infected pigs is often high in newborn pigs, it is less severe in 2–4 week old pigs (Stevenson et al., 2013) and not expected in weaned pigs.

Barrier integrity of the intestinal epithelium has several components, including structural protein–protein interactions between cells of the epithelium (Blikslager et al., 2007) and mucosal surface components (Kim and Ho, 2010) that lubricate and serve as a biological sieve to block foreign material and allow nutrients to the epithelium. The mechanisms by which PEDV antagonize specific functions of the gastrointestinal tract, such as digestion, nutrient absorption and ion gradients, are not well described in nursery pigs. Further, these functions and intestinal integrity are already dramatically altered at weaning (Boudry et al., 2004; Smith et al., 2010) which may exacerbate the impact of PEDV in post-weaned nursery pigs.

There are several publications on molecular characterization of PEDV (Huang et al., 2013; Wang et al., 2014) and host immune response to the virus (de Arriba et al., 2002a,b; Zhang and Yoo, 2016). However, the physiological impact of PEDV on pigs has largely been limited to research focused primarily on suckling pigs (Jung et al., 2006, 2015b) and *in vitro* models (Kim and Lee, 2014a). In addition, the majority of published data has focused on the impact of PEDV during peak infection, but not on restitution or recovery. We have previously shown that PEDV reduces long-term nursery pig growth performance (Curry et al., 2017). Understanding the impact of PEDV on intestinal integrity and function during peak infection and restitution are important in order to design mitigation strategies that may be able to ameliorate or decrease the negative impact this virus has on pig production. The authors hypothesize that initial PEDV infection would

☆ Funding for this research was provided by the Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24574 and Animal Health Hatch funding from the USDA National Institute of Food and Agriculture.

* Corresponding author at: Department of Animal Science, Iowa State University, 806 Stange Road, Ames, IA, 50011, USA.
E-mail address: smcurry@iastate.edu (S.M. Curry).

http://dx.doi.org/10.1016/j.vetmic.2017.09.021
Received 7 July 2017; Received in revised form 27 September 2017; Accepted 27 September 2017

reduce structural intestinal integrity components, but this may coincide with increased mucin production to aid in barrier defense. In addition, modulation of barrier function due to PEDV may continue past the infection period. Therefore, the objective of this study was to investigate the longitudinal impact of PEDV on nursery pig intestinal integrity and function.

2. Materials and methods

2.1. Animals and experimental design

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC# 11-14-7903-S). A total of 50 approximately 5-week-old, maternal line Choice Genetics Large White Pureline Line 3 barrows and gilts (BW = 9.92 ± 0.49 kg) were obtained from a commercial source. Pigs were from a porcine respiratory and reproductive syndrome virus (PRRSV) negative and PEDV naïve herd as determined by routine sow and piglet fecal and blood diagnostics. The specific piglets used and sows were also screened the week prior to selecting the pigs for this study. Animals were randomly allotted to treatment after stratified by BW and sex, with 2–5 pigs per pen and 8 pens per treatment. Treatments were: 1) PEDV naïve, negative control or 2) PEDV inoculated. All pigs were housed in the same facility, but in separate rooms with appropriate biosecurity and biocontainment to prevent cross-infection between groups. On dpi 2, 5, 7, and 14, 4 pigs per treatment were euthanized and tissues were collected. The remaining pigs were used to determine growth performance and tissue accretion over 42 days which is reported elsewhere (Curry et al., 2017).

Pigs were allowed free access to water and a common corn-soybean meal diet formulated to meet or exceed the NRC (2012) requirements for nutrients and energy for this size pig. Diet composition and analysis have been published elsewhere (Curry et al., 2017).

2.2. Inoculation and tissue collection

On dpi 0, PEDV pigs were inoculated via gavage with 5 ml of 10^3 $TCID_{50}$/ml PEDV isolate (USA/Iowa/18984/2013) as previously described (Hoang et al., 2013; Madson et al., 2014). On dpi 2, 5, 7, and 14, 4 randomly selected pigs per treatment were euthanized via jugular sodium pentobarbital (1 ml per 4.5 kg BW) administered via jugular venipuncture. Immediately after euthanasia, intestinal sections were harvested and transported to laboratory as described (Pearce et al., 2013). Briefly, sections from the jejunum (~3 m prior to the ileal-cecal junction) were flushed of luminal contents with Krebs-Henseleit buffer (KB; containing 25 mM $NaHCO_3$, 120 mM NaCl, 1 mM $MgSO_4$, 6.3 mM KCl, 2 mM $CaCl_2$, and 0.32 mM NaH_2PO_4, pH 7.4) and placed into continuously aerated bottles containing KB on ice, to be transported to the laboratory and mounted into modified Ussing chambers (Physiological Instruments, San Diego, CA). The time from euthanasia to mounting into modified Ussing chambers was less than 1 h. Additional adjacent jejunum sections were 1) snap-frozen in liquid nitrogen and stored at −80 °C and 2) fixed in 10% neutral buffered formalin for later analysis. After no more than 48 h, fixed sections were transferred into 70% ethanol for short-term storage before routine processing and paraffin-embedding. The jejunum was used for all analyses in this study as it is one of the primary sites for PEDV infection (Jung and Saif, 2015), and for energy and nutrient digestion and absorption.

2.3. Ex vivo assessment of intestinal barrier function and integrity

Freshly isolated jejunum sections were mounted into modified Ussing chambers to determine transepithelial resistance (TER), macromolecule permeability, and active transport of glucose and glutamine. Briefly, the apparatus was fully assembled with current and voltage electrodes filled with 3% noble agar that were submerged in 3 M KCl, chambers filled with KB, and system leaks were eliminated. Voltage differences between chambers were offset and fluid resistance compensation was used to account for any non-tissue related resistance for so that all chambers measured between 60 and 65 μA.

The serosal layer was removed from the jejunum and sections were pinned onto an insert that allowed for an exposed surface area of 1 cm^2 and then placed into chambers with mucosal and serosal membranes facing opposite chambers. Chambers were constantly aerated with a 95% O_2, 5% CO_2 gas mixture, warmed to 37 °C by circulating water bath, filled with KB, and connected by pairs of current and voltage electrodes to form an electrical circuit. Mucosal and serosal chambers contained 10 mM of D-mannitol and D-glucose, respectively, for ion balance. Sections were voltage clamped at 0 mV and allowed to stabilize for approximately 10–15 min. A pulse current was applied and TER was calculated based on measured voltage and the change in short circuit current when current pulse was applied. The TER was averaged over 10–15 min after stabilization and measured as ohms (Ω) per cm^2. Active mucosal to serosal transport of 10 mM glucose and 10 mM glutamine was determined as previously described (Gabler et al., 2007) and calculated as the difference between baseline and peak response in short-circuit current. Macromolecule permeability was assessed by the mucosal to serosal flux of a 4.4 kDa fluorescein isothiocyanate (FITC)-labelled dextran (FD4, Sigma, St. Louis, MO) as previously described (Mani et al., 2013). A fluorescent plate reader (Bio-Tek, USA) was used to determine relative fluorescence of FD4 in the serosal samples at 485 and 520 nm excitation and emission wavelengths, respectively, and a permeability coefficient was then calculated. Treatment estimates for active transport of glucose and glutamine and Papp were expressed in arbitrary units relative to dpi 2 controls.

2.4. Na+/K+ ATPase and digestive enzyme activity

Na+/K+ ATPase activity in frozen whole jejunum sections was performed as previously described (Pearce et al., 2013). The presence of inorganic phosphate (Pi) which was assessed at 400 nm wavelength using Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Activity of Na+/K+ ATPase was calculated as the difference in Pi production from ATP in the presence or absence of ouabain. Nonspecific phosphate hydrolysis was determined by measuring the liberated Pi in the absence of protein and subtracted from activity measurements. Data are presented as liberated inorganic phosphate (umol) per mg protein per hour.

Activities of lactase, maltase, and sucrase was determined as previously described (Dahlqvist, 1968; Pearce et al., 2013) using freeze-ground jejunum samples. Liberated glucose was measured using the glucose oxidase kit (Sigma, St. Louis, MO) and plates were read at 540 nm wavelength (Synergy 4 microplate reader, Bio-Tek, Winooski, VT). Sample activity for glucose release was compared with a glucose standard and data are presented as liberated glucose (μmol) per minute per gram of protein.

2.5. Caspase 3/7 activity

Executioner caspases (3, 6, and 7) are important for signaling apoptotic events such as DNA degradation, nuclear condensation, and membrane blebbing; therefore, caspase 3/7 activity was determined in jejunum homogenates. A commercially available kit for caspase 3 activity (Cell Signaling, Danvers, MA) was used after proteins were extracted in HEPES buffer (1 M HEPES, 12.5 mM sodium fluoride, 0.64 mM EDTA, and 37.5 mM sodium chloride) with 1% Triton-X and 0.1% protease inhibitor cocktail. Samples were diluted to 1 mg/ml in 1× Assay Buffer and kit instructions were followed without modification. Kit specificity does not distinguish between caspase 3 and 7; therefore, the values determined include activity of both caspase 3 and 7 reported as relative fluorescent unit per minute per gram of tissue.