Porcine Epidemic Diarrhea Virus (PEDV) and Its Regulation of Host Innate Immunity

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AT URBANA-CHAMPAIGN
USA
First PEDV (porcine epidemic diarrhea virus) outbreak in the US, April 2013

Watery diarrhea, Stillborns, ~100% mortality
More than 8 million deaths in 5,267 farms by fall
Clinical signs of PED is indistinguishable from TGEV

Photo: First day outbreak  (Courtesy of Dr. Matt Ackermann via Dr. KJ Yoon)
PEDV Gross lesions:
thin-walled intestine filled with watery material

Intestine is distended with fluid ingesta, and intestinal wall becomes thin and translucent (Courtesy of Dr. Greg Stevenson)
PEDV Confirmed and Presumptive Positive Premises: 39 states are PEDV-positive as of August 1, 2017 - USDA

PEDV Confirmed Positive and Presumptive Positive Premises since June 5, 2014 (confirmed/presumptive) Created: 08/1/2017
Trends in US PEDV Diagnostic Data: PEDV RT-PCR Positive Cases

For the week ending July 08, 2017, 41 positive accessions from 476 submission (8.6% positive). 39 states are confirmed positive since the first outbreak in 2013.
Coronaviruses

Enveloped virus with protruding projections; Spike (S) protein: receptor binding and induction of neutralizing Ab
PED virus is an α-coronavirus (ICTV 2017)

Order **Nidovirales (4 families)**

Family **Coronaviridae (2 subfamilies)**
- **Torovirinae**  
  Berne virus, Breda virus
- **Coronavirinae**  
  (Alpha-, Beta-, Gamma-, Delta-coronaviruses)
  Porcine epidemic diarrhea virus (PEDV)

Family **Roniviridae**  
Okavirus

Family **Mesoniviridae**  
Insect viruses

Family **Arteriviridae (5 genera)**
- **Diparterivirus**  
  Wobbly Possum disease virus
- **Equarterivirus**  
  Equine arteritis virus (EAV)
- **Nesarterivirus**  
  African Oouched rat arterivirus
- **Simarterivirus**  
  Simian hemorrhagic fever virus (SHFV)  
  (10 species)
- **Porarterivirus**  
  Lactate dehydrogenase-elevating virus (LDV)  
  (4 species)
  PRRS virus type 1
  PRRS virus type 2
  Rat arterivirus 1
Likelihood Phylogenetic Trees of the S protein of Coronaviruses: PEDV is an Alphacoronavirus, group b

Wang et al (2017 June), JVI 00764-17, online published
Enveloped with the large genome (26-32 kb) of positive-sense RNA.
Phylogeny of PEDV based on the S gene nucleotide sequences

Lv et al (2016) Virus Adaptation Treatment. 8: 1
PEDV infection requires trypsin for the S protein cleavage
Pathogenesis of TGE

- **ingestion of virus**

- Virus resistant to low pH in stomach and trypsin digestion in intestine

- Following ingestion and 6-12 hours in the stomach, viral replication takes place in the villus epithelium of the jejunum and ileum

- Normal intestinal lumen with prominent villi

- Intestinal villi atrophy
Histopathology: Villous atrophy

Uninfected healthy gut

PEDV-infected gut

(Courtesy of Dr. Paulo Arruda)
Enteric viruses infecting villous enterocytes; PEDV, TGEV, RV

RV infects mature villous enterocytes of mid to distal small intestine

PEDV infects all villi and sporadically infects crypt enterocytes

TGEV infects all villous enterocytes of entire small intestine

Villus

Lamina propria

Crypt

Peyer's patch

M cells

Mesenteric lymph node

Pathogenesis of TGE

Intestinal columnar cells are destroyed by the virus resulting in VILLUS ATROPHY

In an attempt to repair --> immature epithelial cell migrate from the intestinal crypts

These cells have an impaired ability to produce certain digestive enzymes (e.g. lactase, acid phosphatase) and are deficient in ATPase activity, resulting in $\text{Na}^+$ and $\text{K}^+$ imbalances

The presence of undigested lactose within the lumen and alterations of ionic transport are the major contributors to the clinical signs of DIARRHEA and DEHYDRATION
Immunity to PEDV

- IM immunization: humoral IgG - no protection
- Secretory IgA - important in protective immunity and viral clearance
- Oral immunization: develop IgA in mucosal secretions - protective
- Protective IgA in colostrum
- Cell mediate immunity is also important
- High level of interferon production by infected intestinal cells - role in controlling viral replication?
Common Mucosal Immune System

**Effector sites:**
gut and mammary gland

**Live oral vaccines in naïve sows**
Stimulate IgA antibodies to enteric pathogens in gut and mammary gland (milk)

**Gut-mammary sIgA axis**

**Mammary glands**

**Inactivated/subunit IM vaccine in orally primed sows**
Boost IgA and IgG antibodies in gut, mammary gland, and blood
IgA memory B cells reside in gut and spleen (systemic)

**Induction site of oral vaccine or natural infection: gut**

**Spleen**

**IgG**

**IgA**

**Peyer's patches**

**Intestinal villi**

**Mesenteric lymph nodes**

**Thoracic duct**

**Peripheral blood**

**Chattah KS, et al. 2015.**

Piglets will be protected during the first few days by passive antibodies acquired through colostrum and milk. When lactogenic immunity wanes, infected piglets will amplify virus and shed very high levels in their feces and transmit disease to the other piglets in the litter in spite of lactogenic immunity.
Effect of vaccination on neutralizing activity in colostrum and milk in immune sows (A) and native sows (B)- More than one vaccination is needed

Murtaugh et al. 2017 (Aug) Published in Viral Immunology
Summary 1

- Disease caused by enteric viruses infecting villous enterocytes (PEDV, TGEV, RV) can be prevented by local gut immunity

- Lactogenic immunity is required for protection of newborn piglets

- Live replicating virus vaccines may be a choice of effective to achieve full protection

- Multiple vaccination of sows may be need to achieve full protection
# Commercial Vaccines for PEDV

<table>
<thead>
<tr>
<th>PEDV</th>
<th>USA</th>
<th>Inactivated vaccine</th>
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<tr>
<td></td>
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<td>Recombinant alphavirus-based vaccine (RNA-based)</td>
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<td>Europe</td>
<td>Inactivated vaccines</td>
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<td>Inactivated bi-valent TGEV and PEDV vaccine (China; strain <strong>CV777</strong>);</td>
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<td>Asia</td>
<td>Live attenuated tri-valent TGEV, PEDV and porcine rotavirus (China; PEDV strain <strong>CV777</strong>);</td>
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<tr>
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<td></td>
<td>Live attenuated vaccine</td>
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<td>- Japan, PEDV strain 83P-5;</td>
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<td>- South Korea; strains <strong>SM98-1</strong> and <strong>DR-13</strong>;</td>
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<td>- Philippines, strain DR13</td>
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<td>Inactivated vaccine (South Korea, strain <strong>SM98-1</strong>)</td>
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Gerdts and Zakhartchouk (2017, July) Vet Microbiol. 206:45-51
Porcine respiratory coronavirus (PRCV)

PRCV evolved from TGEV (enteric) as a mutant (1984 EU; 1989 USA)

A small deletion in the S protein (S regulates enteric or respiratory tropism > change in cell tropism

Cell tropism

- Respiratory epithelium and alveolar macrophages
- GI epithelium: limited

Generally mild respiratory disease or asymptomatic

*Cross-protection against TGEV
S gene deletion of TGEV and PRCV strains

PRCV induces cross-protection against TGEV. However, diagnosis of PRCV is a challenge. Both PRCV and TGE share many antigenic sites, and so routine serology does not distinguish between the two viruses. Suspicion of PRCV may be based on serological evidence in the absence of enteric disease (difficult).
Approach to generating live-attenuated PEDV (1): Deletions in S1 and ORF3 of cell-culture passaged PEDV in Vero

PC21A: highly virulent PEDV
TC-PC177: Vero cell passaged PEDV- deletion of 197 aa in S1 and 5 nt in ORF3
icPC22A: infectious clone of PC21A
icPC22A-S1del197: Infectious clone of TC-PC177

Modified, Hou et al. 2017 (July) J. Virol; 91:e00227-17
Characterization of S1-Δ197 Recombinant PED Virus

Modified, Hou et al. 2017 (July) J. Virol; 91:e00227-17
Evaluation of the virulence of recombinant viruses icPC22A and icPC22A-S1Δ197 in 5-day-old Gn piglets.
Immunohistochemistry of PEDV N protein in the jejunum sections of piglets that died or were euthanized at 2 to 3 dpi

Modified, Hou et al. 2017 (July) J. Virol; 91:e00227-17
Three-dimensional structural analyses of the S proteins of icPC22A and icPC22A-S1Δ197
Other S gene variant of PEDV with a large deletion

Approach to generating live-attenuated PEDV (2): Deletion of ORF3 gene by Targeted RNA Recombination

ORF3-deleted PEDV

- ORF3 deletion is possible and can generate infectious PEDV
- ORF3-deletion PEDV has not been examined for virulence in pigs

Li et al (2013) PLoS One, 8:e69997
PEDV variants with deletions in the end of S gene and ORF3

Deletion in S and ORF3 of KNU-141112 and viral attenuation

Summary 2: MLV PEDV Vaccine candidates

- Continuous cell culture passages of PEDV will generate deletion mutants in S1 portion and ORF3 gene.

- A deletion of domain 0 in S1 portion of the S protein confers viral attenuation

- Deletion of ORF3 may also confers PEDV attenuation

- For inactivated PEDV vaccines, high titer virus will be helpful

- Expression of S protein is an alternative approach to make inactivated vaccines

- Efficacy and safety test in animals
Nearly all nucleated cells can produce IFN-β through activation of IRF3 and NF-κB.

The IFN-α subtypes are primarily produced by leukocytes.

Plasmacytoid dendritic cells (pDCs) are the most potent type I IFN producers with up to 100 to 1000 times more than other cell types.

The activated pDCs migrate to the lymph nodes and potentially influence various cell types through the secreted type I IFNs.
Anatomy of the intestinal immune system

intraepithelial lymphocytes (IELs): lymphocytes that are activated and migrated up from crypts at the base of the intestine villi to the tip.
IFN-λ determines the intestinal epithelial antiviral host defense

Johanna Post*, Tanel Mahlaköök, Markus Mordstein, Claudia U. Duerr, Thomas Michieletto, Silvia Stockinger*, Peter Staeheli, and Mathias W. Hornet

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RESEARCH ARTICLE

Leukocyte-Derived IFN-α/β and Epithelial IFN-λ Constitute a Compartmentalized Mucosal Defense System that Restricts Enteric Virus Infections

Using Mx1 protein accumulation as marker for IFN responsiveness of individual cells, we demonstrate that intestinal epithelial cells, which are the prime target cells of rotavirus, strongly responded to IFN-λ but only marginally to type I IFN in vivo. Systemic treatment of suckling mice with IFN-λ repressed rotavirus replication in the gut, whereas treatment with type I IFN was not effective.
Interferon-λ and interleukin 22 act synergistically for the induction of interferon-stimulated genes and control of rotavirus infection

Pedro P Hernández1,2,3, Tanel Mahlakõiv4,5,15, Ines Yang6,7, Vera Schwierzeck1,2, Nam Nguyen2, Fabian Guendel1,2,8, Konrad Gronke1,3,8, Bernhard Ryffel9,10, Christoph Hülsmacher12,13, Laure Dumoutier14, Jean-Christophe Renault14, Sebastian Suerbaum6,7, Peter Staehel14 & Andreas Diefenbach1,2,8

The epithelium is the main entry point for many viruses, but the processes that protect barrier surfaces against viral infections are incompletely understood. Here we identified interleukin 22 (IL-22) produced by innate lymphoid cell group 3 (ILC3) as an amplifier of signaling via interferon-λ (IFN-λ), a synergism needed to curtail the replication of rotavirus, the leading cause of childhood gastroenteritis. Cooperation between the receptor for IL-22 and the receptor for IFN-λ, both of which were 'preferentially' expressed by intestinal epithelial cells (IECs), was required for optimal activation of the transcription factor STAT1 and expression of interferon-stimulated genes (ISGs). These data suggested that epithelial cells are protected against viral replication by co-option of two evolutionarily related cytokine networks. These data may inform the design of novel immunotherapy for viral infections that are sensitive to interferons.
Human interferon (IFN) proteins

13 subtypes for IFN-α
Single type for IFN-β
IFN-ε
IFN-κ
IFN-ω

IFN-λ1 (IL-29)
IFN-λ2 (IL-28A)
IFN-λ3 (IL-28B)
IFN-λ4 (recent)
Interferons and intestinal innate antiviral defense

Interferons (IFNs)

I: IFN-α/β
II: IFN-γ
III: IFN-λ

Type III IFNs mainly target mucosal epithelial cells for protection.
IFN-λ plays an important role in PED pathogenesis?

1. PEDV mainly targets IECs in vivo.

2. IFN-λ determines the intestinal epithelial antiviral host defense.
   - IECs potently produce IFN-λ, but not type I IFN.
   - IECs selectively respond to IFN-λ. (No receptor expression for type I IFN)

IECs are the critical cell type responding to IFN-λ to control enteric viral infections.

Hypothesis:

PEDV inhibits the type III IFN responses in intestinal epithelial cells to survive better during infection.

Questions:

1. Can we establish an IEC models for PEDV?
2. Does PEDV induce the production of type III IFN?
3. What is the molecular basis for PEDV-innate?
Cell lines permissive for PEDV

Bright field

PEDV N

Vero Monkey kidney
MARC-145 Monkey kidney
LLC-PK1 Porcine kidney
ST Swine testicle
CΔ2– Swine monocyte
IPEC-J2 Porcine intestinal epithelial

(Dr. A. Blikslager
NC State University)
IPEC-J2 is non-permissive for PEDV: Development of IPEC-DQ porcine intestinal epithelial cells as permissive for PEDV infection.

Dr. A. Blikslager
NC State University

IPEC-J2 → ~20 passages → serial dilution → ~100 cells/well

IPEC-DQ

Expanded in 48-, 24-, 12-, 6-well plate- and large dishes.
IPEC-DQ is a line of porcine intestinal epithelial cells, permissive for PEDV, and produces IFN-λ1, IFN-λ2, IFN-λ4 (IFN-λ2 is deficient in pigs)

**Bright field**

**PEDV N**

IPEC-DQ

IPEC-J2

**IFN-α**
**IFN-β**
**IFN-λ1**
**IFN-λ3**
**IFN-λ4**

0
1
2
3
4
5
1500
1600
1700
1800

Relative IFN mRNA fold change

IPEC-DQ

1 μg/ml trypsin

**Epithelial**
**Intestinal**

**pan Cytokeratin**
**Sucrase-Isomaltase**

**LLC-PK1**

**PEDV**

−     +   −     +

**β-actin**

**Epithelial**

**Intestinal**
IFN-λ is a potent antiviral cytokine restricting PEDV replication.
Luciferase reporter assay for the study of IFN-inhibition

Relative promoter activity = Firefly Luciferase activity / Renilla luciferase activity
PEDV inhibits IFN-λ production
Peroxisomal MAVS selectively activates IRF1-mediated IFN-λ production in intestinal cells

PEDV impairs biogenesis of peroxisomes in IECs

PEDV unimpairs biogenesis of peroxisomes in IECs
Identification of type III IFN antagonists for PEDV
A pig intestinal epithelial cell model has been established for PEDV.

Type III IFN restricts PEDV replication.

In turn, PEDV impairs the peroxisomal biogenesis and suppresses IRF1-mediated type III IFN production.
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