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FULL PAPER

Porcine epidemic diarrhea virus infection in the United States: Etiology, epidemiology, pathogenesis and immunoprophylaxis

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Introduction

Porcine epidemic diarrhea virus (PEDV), a member of the genus *Alphacoronavirus* in the family *Coronaviridae*, causes acute diarrhea/vomiting, dehydration and high mortality in seronegative neonatal piglets (1). The fecal–oral route is the main means of PEDV transmission, although aerosolized PEDV remains infectious (2). For the last three decades, PEDV infection has resulted in significant economic losses in the European and Asian pig industries, but in 2013–2015 the disease has also been reported in the United States (US), Canada, Mexico, Peru, Dominican Republic, Columbia, and Ecuador. The PED epidemic in the US, from April 2013 to 2014, has led to the loss of more than 10% of the US pig population. Since 2013–2014, the newly emerged porcine deltacoronavirus (PDCoV) (genus *Deltacoronavirus*), genetically and antigenically distinct but clinically and pathologically similar to PEDV (3–5), have also spread nationwide and influenced PEDV-infected pigs, hindering the control of either viral infection—another concern raised in US swine (6, 7). However, the impact of dual PEDV and PDCoV infection on the disease outcome in pigs needs to be delineated.

Molecular epidemiology of PEDV in the US

The original US PEDV strains identified during the initial outbreak in 2013 were closely related genetically to the Chinese strains (China/2012/AH2012) reported in 2011–2012, indicating emergence of AH2012-like Chinese PEDV strains in the US (8, 9). The US-like PEDV strains were also found in diarrheic piglets in Japan, South Korea and Taiwan during late 2013 and late 2014 (10–12). Since 2013–2014, other novel PEDV variants have also been found throughout the country. For $<$ 1 year since the first outbreak, the novel US PEDV strains (OH/OH851) with multiple deletions and insertions in their S gene (so called S INDEL strains), which clustered closely with Chinese strain HBQX-2010 or CH/ZMZDY/11, rather than AH2012, were found to possess low nucleotide identity in their 5′-end S1 region (first 1,170 nucleotides) and high nucleotide identity in the remaining S gene, compared to the original US PEDV strains (13, 14). Possible recombination events involving strain(s) from

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China may have contributed to a rapid evolution of US PEDV and the emergence of multiple variants, complicating the molecular epidemiology of US PEDV strains (15). Meanwhile, US-like S INDEL strains have been globally found in Japan, Korea, France, Belgium, and Germany (12, 16–19); however, the cause of global emergence of the certain PEDV strain remains unclear. Recently, another novel PEDV variants originated from a recombination event between an S INDEL and an original US PEDV strain, such as USA/Minnesota211/2014 strain, have been also identified in US swine. The recombinant strains contained the characteristic S INDEL deletions and insertions in the S1 region (20).

Since 2013–2015, the original US PEDV, S INDEL, and recombinant strains have been simultaneously and continuously detected so that they have the potential of seasonal outbreak or epidemic in US swine, although the detection of PEDV-positive cases has been decreased. Based on an epidemiological observation in German farms with epidemic PED caused by US-like S INDEL strains, they appeared to be virulent to cause severe diarrhea, lesions and dehydration, and frequent deaths of neonatal piglets (16). Similarly, a recent study reported that an S INDEL strain Iowa106 is enteropathogenic in neonatal pigs, but with a milder virulence than that of the original US PEDV strain PC21A (21). However, a further comprehensive understanding of the pathogenic characteristics of these strains, including the Minnesota211-like recombinant virus of the original US PEDV and S INDEL strains, is needed.

Disease mechanisms and pathogenesis of PEDV

Disappearance and re-emergence of epidemic PED indicates that PEDV is effectively able to escape from the current vaccination protocols, biosecurity and control systems. Endemic PED is a significant problem, which is exacerbated by the emergence or potential importation of multiple PEDV variants into countries. Epidemic PEDV strains, such as the original US PEDV (non-S INDEL) strains, spread rapidly and cause a high number of pig deaths and substantial economic losses (22). These strains acutely infect villous epithelial cells of the entire small and large intestines although the jejunum and ileum are the primary sites of infection (23, 24). Initial infection and replication of PEDV in the villous epithelial cells, frequently affecting the entire villous epithelium, and subsequent vacuolar degeneration and necrosis of infected cells appeared to acutely occur for 12–24 hours after exposure (25, 26), accompanied by occurrence of proliferating crypt cells to replace the necrotic enterocytes shed at the villi for the same period (25). PEDV infections cause acute, severe atrophic enteritis accompanied by viremia (viral RNA) that leads to severe diarrhea and vomiting, followed by extensive dehydration and imbalanced blood electrolytes as the major cause of death in nursing piglets (1), although the severity of PED may depend on the host (pig age, immunity, etc.) and viral (PEDV strain, exposure dose, etc.) factors. PEDV antigens were evident in the villous or, occasionally, crypt epithelial cells of the small and large intestines and antigen-presenting cells in Peyer's patches, mesenteric lymph nodes, and spleen (27), whereas they were not detected in other organs, such as liver and kidneys. A study reported the replication of PEDV in porcine pulmonary macrophages *in vitro* and *in vivo* (28); however, whether extra-intestinal replication of PEDV occurs still remains uncertain.

Age-dependent resistance to PED

PED is the most devastating in nursing piglets causing 100% morbidity and 50–100% mortality (22, 29). The several mechanisms by which PEDV infection induces greater disease severity and deaths in nursing versus weaned pigs, have been defined (25, 30). Studies reported

that compared to 9-day-old nursing pigs that began to show severe clinical disease and villous atrophy and fecal virus shedding at 1 day after inoculation, a longer incubation period of PEDV was required for 26-day-old weaned pigs to show fecal virus shedding (by 1 more day) or lesions and clinical disease (by 2 more days) (25, 30). There was an innate immune deficiency in functional natural killer cells in the ileum and blood of the nursing piglets that may have contributed to the greater susceptibility of nursing pigs to PEDV infection, compared to weaned pigs (30). Several anatomical and physiological factors that may influence a longer recovery from disease include: 1) the slower turnover of enterocytes (5–7 days) in neonatal piglets compared to 2–3 days in 3-week-old weaned pigs (31); and 2) the anatomically less developed large intestine that may increase the vulnerability to dehydration, compared to weaned pigs (25). Third, there was also a lack of crypt stem cells (LGR5+ cells) and lower numbers of proliferating crypt cells (Ki67+ cells) in the small intestine of nursing pigs, compared to weaned pigs (25). This could lead to slower turnover of enterocytes in nursing vs weaned pigs, contributing to a slower recovery from PED in nursing piglets and the greater susceptibility of nursing pigs to PED (diarrhea and dehydration).

Attenuation of PEDV

The pathogenicity of epidemic PEDV strains is commonly severe, as evidenced by a high mortality of infected nursing piglets. However, attenuation of the virulence of PEDV strains has been induced through high cell-culture passages (93rd–144th) (32–35). The attenuated PEDV strains have multiple nucleotide changes in their S and open reading frame 3 (ORF3) genes compared to those of their parent wild-type strains (32, 34, 35). Among the 652 nucleotides of ORF3, two deletions and seven changes were identified between the parent wild-type DR13 PEDV and the cell-adapted PEDV (100th) that was confirmed to be attenuated (32, 34). Notably, the S genes of the two attenuated PEDV strains, Korean DR13 (100th) and Japanese 83P-5 (100th), had a remarkable similarity with comparable nucleotide mutations and aa substitutions relative to their parental viruses. The attenuated 83P-5 had 18 nucleotide mutations and 13 predicted aa substitutions in the S gene. In contrast, the aa change positions in the S genes of Korean DR13 (100th) and Chinese YN144 (144th) were not comparable (35). The Korean DR13 and Japanese 83P-5 strains are genetically closed to the prototype CV777, whereas the YN strain as a Chinese PEDV variant is genetically less related to CV777 strain (but closed to the original US PEDV strains). The dissimilar selection pressure might be associated with different PEDV strains and cell culture conditions used in these studies.

The sequence analysis of a US PEDV strain and *in vitro* passaged virus (10th in MARC-145 cells) also showed that the cell culture adaptation specifically modifies PEDV S protein (six aa substitutions) whereas the open reading frame 1a/b (ORF1a/b)-encoded polyprotein, ORF3, E, M, and N proteins remained unchanged (36). Multiple nucleotide mutations and aa substitutions in the S gene of PEDV might contribute to attenuation of its *in vivo* pathogenicity, but the entire PEDV genomes should be sequenced to verify other changes after attenuation.

The *in vivo* pathological and immunological mechanisms by which attenuated PEDV successfully infects and replicates in the gut of pigs without causing extensive necrosis of infected cells and clinical disease and then subsequently, the infected cells can be recognized and processed by antigen-presenting cells, need to be defined. There was no detectable clinical signs in four 12-week-old pigs orally inoculated with the Japanese 83P-5 (100th) [$10^{8.2}$ 50% tissue culture infectious dose ($TCID_{50}$)/pig]; however, 3 of the 4 inoculated pigs exhibited fecal virus

shedding (32), implying PEDV released from infected cells possibly via a cell death mechanism. However, whether fecal virus shedding is incident in attenuated PEDV-challenged pigs needs to be further studied, although no intestinal lesions are accompanied. On the other hand, there was no detectable clinical signs and fecal virus shedding in four 10-day-old pigs challenged with the Chinese YN144 (144th) (10^6 $TCID_{50}$/pig); however, small numbers of PEDV antigen-positive enterocytes were found in the small intestine (35). Collectively, the clinical disease, fecal virus shedding, and histopathology (intestinal lesion/PEDV antigen) in challenged pigs should be all evaluated to determine if highly cell-culture passaged PEDV becomes attenuated *in vivo*. In terms of the pathogenicity only, the condition required for the most ideal PEDV vaccine strain is that no seronegative pigs of any ages, especially neonatal piglets, challenged and then, serially passaged with high doses of attenuated PEDV should develop clinical disease and intestinal lesions.

Immunoprophylaxis as a preventive strategy

Immunoprophylaxis as a preventive strategy against epidemic or endemic PED was well-documented in previous reviews (1, 29). In the US, currently, a killed virus, adjuvanted vaccine from Zoetis, Inc. and a RNA particle-based vaccine from Harrisvaccines, Inc. were conditionally licensed to immunize pregnant swine (IM, 2 doses at 2 or 4–5 weeks before farrowing) to protect seronegative breeding farms from epidemic PED; however, no efficacy of these vaccines in experimental or field conditions are reported. Globally, pregnant swine have been vaccinated using live attenuated PEDV strains via an intramuscular or oral route, but induction of complete protection has never been observed in the nursing piglets—the real challenge in securing seronegative farms from epidemic PED or minimizing PEDV-related mortality in seropositive (PEDV-affected) farms as well. Moreover, whether the live vaccine strain is genetically stable and remains non-infectious in the fields needs to be further studied. To select optimal US vaccine candidate strain(s), studies are needed to define whether there are antigenic cross-reactivity between the original US and S INDEL strains, although the antigenic variation between the two strains is expected (37), and their genetic variants (Minnesota211-like) concurrently circulating in US swine and cross-protection among pigs infected by these different strains. In general, a comprehensive understanding of the pathogenic characteristics of epidemic or endemic PEDV strains and possible cross-protection against PED caused by these strains is also needed to prevent and control the disease related to PEDV in affected regions and to develop an effective vaccine.

Conclusions

High mortality of PEDV-infected, seronegative nursing piglets is most likely associated with extensive dehydration as a result of severe villous atrophy. In infected nursing piglets, there is an increased proliferation of crypt cells as well as numbers of LGR5+ crypt stem cells in the intestine, reorganization of the damaged intestinal epithelium, and migration of mature enterocytes to the tips of villi which may be not sufficient to prevent severe dehydration in nursing piglets. The time taken until dehydration of PEDV-infected nursing piglets in the field appears to be too short to enable the animals to recover from the disease through naturally occurring epithelial cell renewal by crypt stem cells. Pharmacological or biological mediators such as epidermal growth factor that promote stem cell regeneration or maturation would also be interesting targets to try to shorten the time for epithelial cell renewal. Combined use of preventive (vaccination) and therapeutic interventions would synergistically reduce PEDV death losses from dehydration and enhance recovery from PED.

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