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Citation	Japanese Journal of Veterinary Research, 64(Supplement 1), S15-S18
Issue Date	2016-02
DOI	10.14943/jjvr.64.suppl.s15
Doc URL	http://hdl.handle.net/2115/61025
Type	bulletin (article)
File Information	02_Zhi Chen.pdf



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Recent outbreaks of PED and its pathogenicity in China

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Received for publication, January 20, 2016

Introduce

Porcine epidemic diarrhea (PED), a serious and highly contagious swine disease, is characterized by severe enteritis, vomiting, watery diarrhea and dehydration in sucking piglets (Chasey and Cartwright, 1978; Pensaert and de Bouck, 1978). The etiologic agent, porcine epidemic diarrhea virus (PEDV), is an enveloped, single stranded positive-sense RNA virus. PEDV infections have substantial detrimental effect on the swine industry because the mortality rates are high, especially in sucking piglets. The major structural gene of 28-kb PEDV encodes the multifunctional virulence factor, spike protein (S), which plays a pivotal role in cell adsorption, membrane fusion and induction of neutralizing antibodies (Bridgen *et al.*, 1993; Pensaert *et al.*, 1981).

1 The origin of new variant PEDV in China

In 1973, the first confirmed PED case in People's Republic of China was reported. Almost 2 decades later, bi-combined killed or attenuated vaccines have been used to against TGEV and PEDV infection in China. The prevalence of PEDV infection was relatively low with only sporadic outbreak. Many farms didn't use the inactivated vaccine at all, and the vaccine production was been halted for a very long time.

However, starting in the October 2010, a remarkable increase in PED emerged in China in an outbreak characterized by high mortality rates among suckling piglets (Pan *et al.*, 2012; Sun *et al.*, 2015). It was reported that just in southern China the outbreak overwhelmed >10 provinces and >1,000,000 piglets died (Sun *et al.*, 2012). This outbreak was distinguished by about 100% illness among piglets after birth (predominantly within 7 days and sometimes

within only a few hours) and death rates of 80%–100%. The affected pigs exhibited watery diarrhea, dehydration with milk curd vomitus and thin-walled intestines with severe villus atrophy and congestion. The disease breaks as early as two days-old, and progressed to death with a few days. Pigs of all ages were affected and exhibited diarrhea and loss appetite with different degrees of severity, which determined to be age dependent; 100% of suckling piglets became ill and death at the end. Pigs over 2 weeks of age experienced mild diarrhea and anorexia, which completely resolved with a few days. Even pregnant sows were affected and exhibited degrees of diarrhea and inappetite. The new PEDV strains which caused such clinical sign described as before were named new variant strains.

The first report on new variant PEDV outbreak was in Thailand. Puranaveja (Puranaveja *et al.*, 2009) reported that in late 2007, PED first outbreak in Nakornpathom province and then spread throughout Thailand. The clinical signs such as watery diarrhea, dehydration, milk curd vomitus and high mortality rate in suckling piglets; mild diarrhea and anorexia in boars and sows were similar to Chinese isolates. Researcher sequenced the M gene of 31 PEDV isolates obtained in Thailand and found that the M gene was highly conserved. The highest sequence identity with Chinese strain, JS2004-2, was 99.2%–99.7%.

Do (Do Tien Duy *et al.*, 2011) reported that in early 2009 emerging PED outbreaks in southern provinces of Vietnam, which is featured by acute diarrhea syndrome in all ages group of pigs, severe watery diarrhea, dehydration, high morbidity and mortality. M gene of virus sequence and phylogenetic analysis showed that the Vietnamese PEDV isolates are “extremely” related to JS-2004-2 and the Thai isolates are “absolutely” related to the Korean isolate (CPF299), because they are identical in nucleotide and amino acid of M gene.

Pan inferred that three Chinese field isolated

strains (CHGD-01, BJ-2011-1 and CH/FJND-3/2011) in 2011 formed a unique cluster with the highest amino acid identities to KNU-0802. The same amino acids insertions and deletions of the S gene were observed among these three Chinese and two Korean (KNU-0802 and KNU-0902) strains. In view of the fact that KNU-0802 and KNU-0902 isolated in South Korean during 2008–2009, earlier than Chinese PEDV breakout, Pan pointed out that the recently isolated Chinese strains may have originated from Korean isolates.

2 The pathogenicity of new variant PEDV

Although most sow herds previously had received CV777-based inactivation or live vaccine, a large-scale outbreak of PED has been associated with rates of illness and death in suckling piglets in China. Sum of the reports on this variant PEDV, the clinical signs can be concluded as follow: pigs of all ages are sensitive to this virus, and exhibited clinical sign of diarrhea and inappetite, but the degree of clinical signs varied by their ages. Boars and sows had mild diarrhea and anorexia for a few days and recovered within a week. 100% infected suckling piglets below 10 day-old-age became ill and death at the end. Pigs over 2 weeks of age experienced mild diarrhea and anorexia, which completely resolved with a few days. The gross pathological lesions were confined to the small intestine and were characterized by thin translucent intestinal walls that contained moderate amounts of yellow watery feces without macroscopic trace of blood. No other gross abnormalities were noticed. Histological evaluation revealed regions of small intestines with villus blunting and fusion and minimal lymphoplasmacytic infiltration of the villi of the lamina propria.

Three PEDV field strains were isolated from different farms where new variant strains infected in our lab (Wang *et al.*, 2013). In order to determine the virulence of the variant strains (ZB, YS and SH), an experiment was performed.

Fifteen 4-day-old Duroc crossbred piglets were randomly allotted to 3 groups, each group consisting of 5 pigs. Piglets orally infected with the YS and ZB isolates. In YS isolate infected group, one piglet died at 5 dpi, and 4 piglets died at 6 dpi. Before death, the piglets showed hemorrhage and shedding in the gastric mucosa, swelling and congestion in the mesenteric lymph nodes, and hemorrhage in the intestinal wall. Histopathology changes included epithelial cell shedding; intrinsic layer hemorrhage and excessive infiltration of lymphocytes in the stomach; and congestion edema, and epithelial cell shedding in the intestinal mucosa. In ZB infected group, diarrhea was observed at 3–5 dpi, and all piglets died at 5 dpi. The dead piglets showed similar lesions to those of the piglets infected with YS isolate in the stomach, intestines, and mesenteric lymph nodes. At last the PEDV was recovered from the dead piglets, and no amino acid mutation was found in S protein. Piglets in control group remains healthy, and no obvious pathologic changes were observed. This investigation indicated that the recent diarrhea outbreaks were mainly caused by PEDV variants.

3 Genetic features of the PEDV variant strains in China

As a member of genus Coronavirus, PEDV is an enveloped, single-stranded, positive-sense RNA virus. The genome is approximately 28 kb in length and encodes four mainly structural proteins, which are the spike (S) protein, the membrane (M) protein, the envelope (E) protein, and the nucleocapsid (N) protein. The Chinese variant PEDV strains are highly virulent in piglets. Li (Li *et al.*, 2012) collected 9 PEDV S gene sequences from pigs at 9 farms, where animals had severe diarrhea and high mortality rate. After aligned with 24 previously published PEDV S genes, it is found that the full length S gene sequences of the 9 isolates showed overall high conservation with the reference strains, up

to 94.9%–99.6% homology. Compared to vaccine strain (CV777), the distinct difference of gene in the new variant strains locates in spike (S) protein. S protein harbors amino acid deletion and insertion. There are two separate insertions in S protein, one is 1-aa insertion at position of 140, and the other is 4-aa insertion at position between 59 and 62. The only identified deletion locates at position of 163 and 164.

Conclusion

Recently, a large number of studies of PEDV have provided an in-depth understanding of PEDV infections in the pig population in China. In particular, the origin of new variant, pathogenicity, and genetic features are clearly understood. These understandings are powerful tool for the control of PED. Further work should continue to trace the variability of PEDV strains, improve PEDV vaccines using variant strains as candidate and elucidate the pathogenic mechanism of PEDV variant strains.

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