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Efficacy of an inactivated genotype 2b porcine epidemic diarrhea virus vaccine in neonatal piglets

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ABSTRACT

Massive outbreaks of porcine epidemic diarrhea virus (PEDV) recurred in South Korea in 2013–2014 and affected approximately 40% of the swine breeding herds across the country, incurring a tremendous financial impact on producers and consumers. Despite the nationwide use of commercially available attenuated and inactivated vaccines in South Korea, PEDV has continued to plague the domestic pork industry, raising concerns regarding their protective efficacies and the need for new vaccine development. In a previous study, we isolated and serially cultivated a Korean PEDV epidemic strain, KOR/KNU-141112/2014, in Vero cells. With the availability of a cell culture-propagated PEDV strain, we are able to explore vaccination and challenge studies on pigs. Therefore, the aim of the present study was to produce an inactivated PEDV vaccine using the KNU-141112 strain and evaluate its effectiveness in neonatal piglets. Pregnant sows were immunized intramuscularly with the inactivated adjuvanted monovalent vaccine at six and three weeks prior to farrowing. Six-day-old piglets born to vaccinated or unvaccinated sows were challenged with the homogeneous KNU-141112 virus. The administration of the inactivated vaccine to sows greatly increased the survival rate of piglets challenged with the virulent strain, from 0% to approximately 92% (22/24), and significantly reduced diarrhea severity including viral shedding in feces. In addition, litters from unvaccinated sows continued to lose body weight throughout the experiment, whereas litters from vaccinated sows started recovering their daily weight gain at 7 days after the challenge. Furthermore, strong neutralizing antibody responses to PEDV were verified in immunized sows and their offspring, but were absent in the unvaccinated controls. Altogether, our data demonstrated that durable lactogenic immunity was present in dams administrated with the inactivated vaccine and subsequently conferred critical passive immune protection to their own litters against virulent PEDV infection.

1. Introduction

Porcine epidemic diarrhea (PED) is a highly contagious and deadly swine disease that is characterized by watery diarrhea, vomiting, severe dehydration, and high mortality rates in neonatal piglets (Lee, 2015; Saif et al., 2012). Although this enteric disease was first recognized in England in 1971, it was not identified until 1978 when a coronavirus was described as the etiological agent of PED (Oldham, 1972; Pensaert and Debouck, 1978). PED virus (PEDV) is a member of the genus *Alphacoronavirus* within the family *Coronaviridae* of the order *Nidovirales* (Pensaert and Debouck, 1978; Lee, 2015). PEDV is a large, enveloped virus that contains a single-stranded positive-sense RNA genome of approximately 28 kb with a 5′ cap and a 3′ polyadenylated tail (Pensaert and Debouck, 1978; Saif et al., 2012). The PEDV genome is composed of a 5′ untranslated region (UTR), at least 7 open reading frames (ORF1a, ORF1b, and ORFs 2–6), and a 3′ UTR (Kocherhans et al., 2001). The two large ORF1a and ORF1b encode two replicase polyproteins (pp), 1a and 1ab, which are later proteolytically processed into mature

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non-structural proteins. The remaining ORFs in the 3′ terminal region code for four major structural proteins, namely, the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (Duarte and Laude, 1994; Lai et al., 2007; Lee, 2015). Among these, the S glycoprotein has been considered an appropriate viral gene for sequencing in order to investigate genetic relatedness and molecular epidemiology of PEDV isolates (Chen et al., 2014; Gerber et al., 2014; Lee et al., 2010; Lee and Lee, 2014; Oh et al., 2014). On the basis of phylogenetic analysis of the S gene, PEDV can be genetically divided into 2 groups: genogroup 1 (G1; classical or recombinant and low-pathogenic) and genogroup 2 (G2; field epidemic or pandemic and high-pathogenic), each of which is composed of two subgroups, 1a and 1b, and 2a and 2b, respectively (Lee, 2015; Lee et al., 2010; Lee and Lee, 2014; Oh et al., 2014).

Although PED has been described in Europe and Asia, the most serious epizootics have occurred predominantly in Asian swine-producing countries over the past two decades. Despite a notorious reputation in Asia, PED was not globally well recognized until the disease struck the United States in early 2013. Since its incursion into the US, PEDV has rapidly spread nationwide and to neighboring countries, sustaining enormous damages in pig health and the pork industry (Mole, 2013; Stevenson et al., 2013; Vlasova et al., 2014). Soon thereafter, severe PED epidemics recurred in South Korea, Japan, and Taiwan, and US prototype-like G2b PEDV strains were responsible for recent outbreaks in these countries (Lee and Lee, 2014; Lin et al., 2014; Suzuki et al., 2015). More recently, PEDV re-emerged throughout western and central Europe (Boniotti et al., 2016; Hanke et al., 2015; Grasland et al., 2015; Mesquita et al., 2015; Steinrigl et al., 2015; Theuns et al., 2015). These re-emergent PEDV strains were phylogenetically similar to new low-pathogenic G1b variants identified first in China and later in the US, South Korea, and Japan (Lee et al., 2014b; Li et al., 2012; Suzuki et al., 2015; Wang et al., 2014). Therefore, PED is now considered an emerging and re-emerging viral disease of swine around the world, leading to significant financial concerns in the global pork business.

The first PED epizootic in South Korea was reported in 1992 (Kweon et al., 1993). Since then PED outbreaks have continually occurred, resulting in substantial economic losses to the domestic swine industry. Moreover, the recent 2013–2014 PED epidemics swept through the national herd and killed hundreds of thousands of piglets across mainland South Korea followed by Jeju Island (Lee et al., 2014a; Lee and Lee, 2014). Meanwhile, all four different genotypes of PEDV are present in South Korea, including vaccine strains (G1a), new variants (G1b), past epidemic strains (G2a), and current dominant epidemic strains (G2b) (Lee, 2015; Lee et al., 2010; Lee et al., 2014b; Lee and Lee, 2014). Although both modified live and inactivated/killed vaccines against PED are commercially available in South Korea, their efficacy in the field is still being debated. The low to moderate effectiveness of current PEDV vaccines may be attributed to antigenic, genetic, and phylogenetic differences between the major S glycoproteins of the vaccine and field epizootic strains (Kim et al., 2015; Lee, 2015; Lee et al., 2010; Lee and Lee, 2014; Lee et al., 2014a; Oh et al., 2014). Considering these issues, G2b epidemic or related strains prevalently circulating in the field should be employed for the development of next-generation vaccines to control PED. However, isolating PEDV in cell culture has proven fastidious, and even the isolated virus may be incapable of retaining infectivity upon further *in vitro* passage. This laboratory hurdle makes the production of efficacious vaccines difficult. Recently, a highly virulent Korean G2b strain KOR/KNU-141112/2014 was isolated in our laboratory and sequentially passaged in cell culture. In the present study, we developed a Korean field isolate-derived inactivated vaccine and assessed its efficacy on suckling piglets against homogeneous PEDV challenge.

2. Materials and methods

2.1. Cells and virus

Vero cells (ATCC CCL-81) were cultured in alpha minimum essential medium (α-MEM; Invitrogen, Carlsbad, CA) with 5% fetal bovine serum (FBS; Invitrogen) and antibiotic-antimycotic solutions (100×; Invitrogen) and maintained at 37 °C in a humidified 5% CO_2 incubator. A Korean PEDV strain, KOR/KNU-141112/2014, was isolated and propagated in Vero cells in our lab as described previously (Lee et al., 2015). A viral stock at the 10th passage in cell culture (KNU-141112-P10) was prepared and used in this study. Briefly, confluent Vero cells grown in 100-mm diameter tissue culture dishes were washed with PBS and inoculated with 1 ml of 10-fold diluted PEDV KNU-141112 containing trypsin (USB, Cleveland, OH). After incubating at 37 °C for 1 h, 7 ml of virus growth medium [α-MEM supplemented with antibiotic-antimycotic solutions, 0.3% tryptose phosphate broth (TPB; Sigma, St. Louis, MO), 0.02% yeast extract (Difco, Detroit, MI), 10 mM HEPES (Invitrogen), and 5 μg/ml of trypsin] was added. The inoculated cells were maintained at 37 °C under 5% CO_2 and monitored daily for cytopathic effects (CPE). When 70% CPE appeared, inoculated cells were subjected to three rounds of freezing and thawing. The culture supernatants were then centrifuged for 10 min at 400*g* (Hanil Centrifuge FLETA5, Incheon, South Korea) and filtered through a 0.45-μm-pore-size filter (Millipore, Billerica, MA). The clarified supernatants were aliquoted and stored at −80 °C as the viral stock until use.

2.2. Virus inactivation

Before inactivation, KNU-141112-P10 virus was purified as described previously (Lee and Lee, 2013), and the purified virus ($10^{7.0}$ $TCID_{50}$/ml) was inactivated for use as a vaccine. Inactivation of PEDV with binary ethylenimine (BEI) was performed as described previously (Vanhee et al., 2009). Briefly, virus was inactivated by the addition of 0.1 M BEI to a final volume of 5% and incubating at 37 °C for 24 h. Excess BEI was then neutralized by incubation with sodium thiosulfate at 37 °C for 2 h. Inactivated virus was stored at −80 °C until use. Virus inactivation was verified by inoculation of Vero cells with the BEI-treated virus. The inoculated cells were analyzed for CPE, followed by immunofluorescent staining with a PEDV N protein-specific monoclonal antibody (Lee et al., 2015).

2.3. Experimental design

Swine vaccination and challenge experiments described here were performed at the Choongang Vaccine Laboratory Animal Facility under the guidelines established by its Institutional Animal Care and Use Committee. A total of 5 commercial crossbred sows (Great Yorkshire × Dutch Landrace) with the same parity and expected farrowing date were chosen at a conventional breeding farm with a high health status and no known prior PED outbreak or vaccination with PEDV. All animals were confirmed negative for PEDV, transmissible gastroenteritis virus (TGEV), porcine deltacoronavirus, and porcine rotaviruses by virus-specific PCRs on rectal swabs and determined to be free of antibodies to PEDV as well as to TGEV and porcine reproductive and respiratory syndrome virus. Pigs were randomly assigned to 2 experimental groups: vaccinated group 1 ($n = 3$; V1, V2, and V3) and unvaccinated group 2 ($n = 2$; C1 and C2). Three sows in group 1 were intramuscularly (IM) vaccinated twice at 6 and 3 weeks prior to farrowing with 2 ml of the experimental vaccine (1 ml of the inactivated virus in a 1 ml water-in-oil adjuvant) containing 10^7 $TCID_{50}$ of the BEI-inactivated PEDV KNU-141112 strain. The remaining two sows in group 2 were not vaccinated and served as controls. At 5 days post-farrowing,