Evaluation of the Efficacy of Lactogenic Immunity of Sow Induced by Porcine Epidemic Diarrhea Virus (PEDV) Vaccine By Intranasal Immunization

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Research Article

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Abstract

Background: Porcine epidemic diarrhea virus belongs to family of coronaviruses which are notorious for rapid spread of severe diarrhea among suckling piglets. The virus mainly replicates in the epithelial cells of duodenum, jejunum, ileum and colon and is a life threatening condition in pigs. A highly virulent strain “CHYJ130330” having high mortality rate was isolated from a field outbreak, identified as a new virulent genotype II/G2-b strain and adapted successfully to vero cells was used to prepare inactivated vaccine against PEDV. This newly prepared vaccine was given through intranasal route and is compared with the commercially available bi-combined (PEDV and TGEV) vaccine given by intramuscular injection. In this study milk or mucosal IgA and IgG antibody levels have been used to predict vaccine efficacy and the level of protective immunity against PED virus. Antibody titers in the milk of sows and intestines of suckling piglets were compared by enzyme-linked immunosorbent assay (ELISA).

Results: It was shown that CHYJ vaccine induced significantly higher levels of PEDV IgA antibody in milk of sows and intestines of piglets as compared to commercial bi-combined vaccine. Both CHYJ and commercial vaccines were not able to induce detectable IgG levels in the intestines of piglets; the later however induced higher IgG levels when detected in the sow’s milk. Protective efficacy of vaccines was determined against a highly virulent PEDV strain. CHYJ intranasal vaccine gives a better protection 80% (4/5) rate as compared to commercial i.m. vaccine conferring 60% (3/5) immunity in suckling piglets.

Conclusions: It is therefore concluded that PEDV inactivated CHYJ vaccine confer better lactogenic immunity and gives more protection to suckling piglets than available bi-combined TGEV and PEDV vaccine through passive immunization.

Introduction

Porcine epidemic diarrhea virus (PEDV) is a highly virulent re-emerging enteric coronavirus that causes porcine epidemic diarrhea (PED) characterized mainly by acute diarrhea, dehydration and death in neonatal piglets [1]. The disease was first reported in Europe in 1971 and later on spread rapidly across Europe and Asia [2]. It was until 2010, PED was recognized as a sporadic enteric viral disease with seasonal prevalence, however, by the end of 2010 there were more severe outbreaks with frequency all the year around reporting 90–100% morbidity and 70–100% mortality in neonatal piglets [3-5]. The virus was first reported in the United States in 2013, causing more than 8 million deaths in suckling piglets in US during one year epidemic period and spread rapidly across swine-growing regions of the United States and the Americas [6-7]. PEDV infection rapidly spreads throughout the gastrointestinal tract [8], allowing virus to replicate in the cytoplasm of villous epithelial cells thereby damaging the mucosal surface of small intestine [9]. In order to prevent this damage, an effective strategy which can protect/enhance the immunity of host mucosal surfaces should be adopted [10]. Currently, traditional vaccines administered via the parenteral route by subcutaneous (s.c.) or intramuscular (i.m.) injection have been widely used, however these approaches did not induce high titers of maternal antibodies as well as PEDV-specific IgA antibodies resulting in inadequate protection to intestinal mucosal surfaces in newborn piglets against
PEDV infections [11-12]. In this way, maternal vaccines to induce lactogenic immunity and their transmission to suckling piglets via colostrum and milk are pivotal for early passive protection [13-14]. Lactogenic immunity until weaning remains the most promising and effective way in protecting suckling piglets against enteric diseases like PEDV [14-15]. This is dependent on trafficking of pathogen-specific IgA+ plasmablasts to the mammary gland (MG) and accumulation of secretory IgA (sIgA) antibodies in milk, named the “gut-MG-sIgA axis” [16-18]. Maternal vaccination that increases the amount of passively transferred protective antibodies through milk, induced via the gut-MG-sIgA axis, is the strategy used to protect suckling piglets from PEDV immediately after birth [14-15]. The increased rate of protection was found to be associated with high titers of IgA antibodies in milk. This demonstrates that enteric viral infection stimulates the intestinal mucosa influencing lactogenic immunity via the gut-MG-sIgA axis [14-15]. Due to the impermeable nature of placenta in sows, piglets are born agammaglobulinic and therefore they are highly susceptible to a plethora of infectious agents. It is due to this reason that neonatal pigs rely solely on colostrum and milk antibodies for passive immunity [15]. In sows, IgG is dominant in colostrum and is transuded from sow serum [19]. Neonatal piglets acquire colostral antibodies (mainly IgG) via nursing. These immunoglobulins are transported across the piglet’s intestinal epithelium only within the first 24-48 hours after birth. During the next 2-3 days, the transition from colostrum to milk, sIgA becomes dominant and persists in milk throughout lactation period. In contrast to the IgA antibodies dominant in milk and function to provide local passive protection to the piglet intestinal tract, its resistance to proteolytic enzymes imparts sIgA, a high level of stability in the gastrointestinal tract. This discovery led to maternal vaccination strategies to induce mucosal passive immunity applicable to multiple species and multiple enteric pathogens, including PEDV. Thus vaccination strategies against PEDV must focus on induction of mucosal immunity to protect the target intestinal enterocytes. This necessitates protective levels of mucosal immunity in neonates at birth and throughout the nursing period.

In this study, inactivated vaccine prepared using a highly virulent field strain “CHYJ130330”, was used to immunize pregnant sows via mucosal route and antibody titers in the form of IgA and IgG were checked in the milk and also in the intestine contents including duodenum, jejunum, ileum and colon of suckling piglets. Challenge studies were also carried out to test the efficacy and protection rate in piglets using this newly developed vaccine.

**Materials And Methods**

**Cells and virus**

The wild-type CHYJ130330 strain (GenBank Accession No. KJ020932) was isolated, sequenced and preserved in our laboratory [20]. The strain was propagated in Vero cells grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 5% fetal bovine serum (FBS; Life Technologies), penicillin (100 units/ml) and streptomycin (100 mg/ml) at 37°C in a humidified atmosphere with 5% CO₂. Briefly, for viral infection, the confluent monolayer of Vero cells propagated in a growth medium in 25 cm² flasks was washed three times with phosphate-buffered saline (PBS, pH7.4)
and inoculated with 1 ml virus. After adsorption at 37°C for 2 hrs, infected cells were maintained in growth medium supplemented with 5% fetal bovine serum at 37°C in 5% CO₂. The 8ᵗʰ generation of PEDV CHYJ strain (containing 10⁷ TCID₅₀) was used for highly pathogenic PEDV challenge experiment in piglets.

**Vaccine**

The inactivated PEDV CHYJ vaccine contained porcine epidemic diarrhea virus at 10⁷.₅ TCID₅₀/ml. The porcine epidemic diarrhea virus venom was added with the final content of 0.2% formaldehyde solution and inactivated at 37°C for 48 hours, and then 1% sterilized sodium sulfite (5%) was added to terminate the inactivation. Porcine epidemic diarrhea virus venom is mixed with 50% sterilized alumina hydroxide gel brine diluent in a ratio of 7:3 to produce the inactivated vaccine. The commercial bi-combined (PEDV and TGEV) inactivated vaccine contained porcine epidemic diarrhea virus CV777 strain.

**Animals, Vaccination and experimental design**

15 pregnant sows tested negative for PEDV antibodies as well as screened for antigen from un-immunized PEDV healthy pigs were randomly allocated three groups. The allocation was done 40 days prior to farrowing with five sows in each group. First group sows (n=5) were intranasally vaccinated with inactivated PEDV CHYJ vaccine 40 days prior farrowing followed by same titer booster dose after 20 days and the dose was two milliliters per head. Second group was vaccinated intramuscularly with commercial bi-combined (PEDV and TGEV) inactivated vaccine, and the dose was two milliliters per head. Along with this vaccine control group, a third negative control group was also kept as shown in Table 1. After farrowing, milk was collected from sows in all groups on day 0, 1, 5, 10, 15 and 21. From each experimental group, five suckling piglets were randomly selected for dissection as shown in Table 2. Intestine contents including duodenum, jejunum, ileum and colon of piglets were collected aseptically before and after offering milk on day 1, 5, 10, 15 and 21. Immunoglobulins (IgA and IgG) were tested in milk (sows) and intestine contents (piglets) using PED IgA Ab ELISA antibody test kit and Porcine Epidemic Diarrhea Virus Antibody Test Kit respectively.

Table 1 Experimental groups, Vaccination and milk of sows collection at different days post farrowing

<table>
<thead>
<tr>
<th>Group designation</th>
<th>No. of sows</th>
<th>Type of Vaccine</th>
<th>Route</th>
<th>Milk collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHYJ vaccine</td>
<td>5</td>
<td>Inactivated PEDV CHYJ vaccine</td>
<td>Intranasally</td>
<td>Collect milk at day 0, 1, 5, 10, 15 and 21 after farrowing</td>
</tr>
<tr>
<td>Commercial vaccine</td>
<td>5</td>
<td>Inactivated commercial bi-combined vaccine</td>
<td>Intramuscularly</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>Saline</td>
<td>Intranasally</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Experimental groups, Vaccination and intestine of piglets collection at different days post farrowing

<table>
<thead>
<tr>
<th>Group designation</th>
<th>No. of piglets</th>
<th>Type of Vaccine</th>
<th>Route</th>
<th>Intestine collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHYJ vaccine</td>
<td>25</td>
<td>Inactivated PEDV CHYJ vaccine</td>
<td>Intranasally</td>
<td>Collect intestine of five suckling piglets including duodenum, jejunum, ileum and colon before offering milk and at day 1, 5, 10, 15 and 21 after offering milk</td>
</tr>
<tr>
<td>Commercial vaccine</td>
<td>25</td>
<td>Inactivated commercial bi-combined vaccine</td>
<td>Intramuscularly</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>25</td>
<td>Saline</td>
<td>Intranasally</td>
<td></td>
</tr>
</tbody>
</table>

Sample collection

Samples including milk (from sows after farrowing) and intestine contents (from suckling piglets) were aseptically collected before milk intake and also after offering milk on day 0, 1, 5, 10, 15 and 21 and stored at -20°C. Equal sections of piglet’s intestine were scraped, collected aseptically and centrifuged at 10,000 rpm. Supernatant obtained was stored at -20°C.

ELISA

PEDV IgA antibody detection was carried out for milk obtained from sows and intestine contents from piglets using PED IgA Ab ELISA antibody test kit. The kit contained microporous plates that have been coated with PED IgA antigen. Samples were loaded to each well including positive (Ab-positive) and negative (Ab-negative) controls in duplicate for each sample type. Plates were incubated at 37°C for 60 minutes and washed 5 times (5’). Diluted enzyme conjugate was dispensed in each well; plates were incubated for 30 minutes. In the next step, the plates were washed 5 times (5’), substrate was dispensed in each well and plates were placed for 15 minutes at room temperature (18~25°C). The stopping solution was added to each well and the absorbance was measured at 630nm using bichromatic spectrophotometer within 30 minutes upon completion of assay.

PEDV IgG antibodies in milk of sows and intestines of piglets were also tested using Porcine Epidemic Diarrhea Virus Antibody Test Kit. The kit contained microporous plates that have been coated with PEDV antigens. Each sample was loaded along with antibody positive (positive control) and negative samples (negative plate control), run in duplicate on each ELISA plate. Calculate the results. The plates were kept at 23±2°C for 60 minutes and washed 4 times (4’) carefully. Diluted conjugate A was dispensed to each well, the plates were kept for 60 minutes at 32±2°C. After washing (4’), diluted conjugate B was added to each well and plates were kept for 60 minutes at 23±2°C. After incubation, plates were washed four times and substrate was dispensed to each well. The plates were kept in dark for 10 minutes at 23±2°C before
dispensing stopping solution to each well. The absorbance was read within 15 minutes at 630nm using bichromatic spectrophotometer.

**Challenge**

The virulent PEDV CHYJ strain was grown to a final titer of $10^2$ TCID$_{50}$/mL. Five piglets were randomly selected from each group and challenged orally with 1 mL of $10^2$ TCID$_{50}$ virulent PEDV CHYJ strain at day 3 post farrowing. Piglets in the negative control group were orally sham-inoculated with 1 mL saline. All piglets were examined daily for clinical signs of illness and anal swabs were also collected each day (day 1 to 5) for detection of PED virus using RT-qPCR.

**Statistical analysis**

All data were expressed as the mean ± standard deviation (SD). IgA and IgG antibody titers were subjected to statistical analysis for estimating the protective levels. GraphPad Prism 5 was employed for correlation analysis and a value of $p<0.05$ was considered statistically significant.

**Results**

**PEDV IgA antibody levels in milk of sows and intestines of piglets**

The PEDV IgA antibodies in the milk of sows immunized with inactivated PEDV CHYJ vaccine were positive at day 0, 1, 5, 10, 15 and 21 after farrowing (OD$_{630} \geq 1.0$). In contrast, the sows in the commercial vaccine group were positive for PEDV IgA antibodies in milk at day 0 and 1 after farrowing (OD$_{630} \geq 1.0$). All sows in the negative control group were negative for PEDV IgA antibodies in milk (OD$_{630} = 0.423$) (Fig. 1). The piglets in all groups were negative for PEDV IgA antibodies in intestines before milk feeding. After milk feeding, IgA antibodies were found to be present in the intestines including the duodenum, jejunum, ileum and colon of the piglets vaccinated with inactivated PEDV CHYJ vaccine. The IgA antibodies were produced starting from day 1 until day 15 after farrowing and later on negative or weakly positive. These antibodies were found mainly distributed in jejunum, ileum and colon of piglets, and to less extent in duodenum (Fig. 2 and 3). The commercial vaccine group after milk feeding became weakly positive for IgA antibodies at day 5 found distributed only in ileum; however, these were not detected at day 10 after farrowing. Neither of duodenum, jejunum and colon were positive or weakly positive for IgA antibodies after farrowing (Fig. 4). All piglets in the negative control group, were found negative for IgA in intestines throughout lactation (Fig. 5).

**PEDV IgG antibody levels in milk of sows and intestines of piglets**

Both experimental i-e, inactivated CHYJ and commercial vaccine, groups successfully stimulate IgG antibody production (Fig. 6). The sows in inactivated PEDV CHYJ vaccine group were having IgG antibodies in milk at day 0, 1 and 5 after delivery, and tested negative at day 10 after farrowing. While the sows in commercial vaccine group were tested positive for IgG antibodies in milk at day 0 and 1 after
farrowing. Although the commercial vaccine produced significantly higher level of IgG antibodies at day 0 and 1, which become negative at day 5; these antibodies in case of CHYJ vaccine group were lower in titer but persisted for a longer time. PEDV IgG antibodies were not detected in sows of the negative control group throughout the entire duration of the study. Piglets of all vaccinated as well as control groups were negative for PEDV IgG antibodies in intestines including the duodenum, jejunum, ileum and colon before or after offering milk (Fig. 7, 8, 9 and 10).

**Piglets challenge**

The protection rate in case of inactivated PEDV CHYJ vaccine group was 4/5, as compared to commercial vaccine group (3/5). The morbidity rate was 100% (5/5) in the challenge control group. All piglets in the negative control group were found normal. The unprotected piglet (1/5) in CHYJ group showed slight diarrhea, 96 hours after challenge and was also found positive for PED virus through RT-qPCR. The other piglets (4/5) in the above group were not having any signs of diarrhea and were also negative for PED virus through RT-qPCR. Two piglets (2/5) in commercial vaccine group showed diarrheal symptoms 96 hours after challenge, confirmed positive for virus and showed thinning of the intestinal wall along with watery consistency of intestinal contents. The other piglets (3/5) were normal throughout the experimental and the result of RT-qPCR were also found negative. Piglets in the challenge control group began to show diarrheal symptoms 48h after challenge, which become severe after 72 hours. There is thinning of wall of intestines and RT-qPCR was also positive for PED virus in these piglets. All piglets (5/5) in the negative control group were normal throughout the experiment. The results are shown in table 3.

Table 3 The PEDV positive rate and protection rate of piglets in each group after challenge experiments.

<table>
<thead>
<tr>
<th>Vaccine used</th>
<th>No. of piglets</th>
<th>PEDV positive rate</th>
<th>Protection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHYJ vaccine</td>
<td>5</td>
<td>1/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Commercial vaccine</td>
<td>5</td>
<td>2/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Challenge control</td>
<td>5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>0/5</td>
<td>—</td>
</tr>
</tbody>
</table>

**The correlation between PEDV IgA and IgG in milk of sows and intestines of piglets**

As shown in Table 3, at day 1 after farrowing, the correlation coefficient value (|r|) of PEDV IgA in milk of sows was 0.750 (P = 0.001) with PEDV IgA in intestines of piglets, while the correlation coefficient value (|r|) of PEDV IgG in milk of sows was 0.379(P = 0.164) with PEDV IgG in intestines of piglets.

Table 3 Spearman’ s non-parametric correlations between PEDV IgA and IgG in milk of sows and intestines of piglets
The correlation between antibody titers and protective rate

As shown in Table 4, at day 1 after farrowing, the correlation coefficient value (|r|) was 0.993 (P = 0.001) with PEDV IgA in milk of sows, 0.993 (P = 0.001) with PEDV IgA in intestines of piglets, 0.500 (P = 0.667) with PEDV IgG in milk of sows, 0.500 (P = 0.667) with PEDV IgG in intestines of piglets. The protective rate of piglets is mainly related to PEDV IgA antibody, not PEDV IgG (Fig. 11).

Table 4 Spearman’s non-parametric correlations between antibody titers and protective rate

<table>
<thead>
<tr>
<th>Protection rate</th>
<th>PEDV IgA in milk of sows</th>
<th>PEDV IgG in milk of sows</th>
<th>PEDV IgA in intestines of piglets</th>
<th>PEDV IgG in intestines of piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEDV IgA in milk of sows</td>
<td>r 0.993</td>
<td>p 0.001</td>
<td>r 0.993</td>
<td>p 0.001</td>
</tr>
<tr>
<td>PEDV IgG in milk of sows</td>
<td>r 0.500</td>
<td>p 0.667</td>
<td>r 0.500</td>
<td>p 0.667</td>
</tr>
</tbody>
</table>

Discussion

Since lactogenetic immunity protects suckling piglets against PEDV infection, induction of passive mucosal immune responses might play an effective role in protecting newborn piglets. It has been shown that maternal vaccination enhances the passively transferred protective antibodies through colostrum and milk, induced via the gut-MG-sIgA axis [14–15]. PEDV specific IgA and IgG antibodies appeared in milk when the late-term pregnant sows were given attenuated PEDV vaccine orally [21–23]. Here, in this study, we evaluated the impact of inactivated PEDV CHYJ vaccine given 40 days prior farrowing to quantify the maternal immune response, particularly generation of PEDV-specific IgA and IgG antibodies in sow’s milk and intestines of suckling piglets. Challenge studies were also carried out using PEDV virulent strain to determine the mucosal protection provided through passive immunization.
In piglets, the extent of protection against PEDV is based on the presence of specific IgA antibodies in the milk of immuned sows [15]. After antigenic sensitization in the gut, IgA immunocytes migrate to the mammary gland, where they localize and secrete IgA antibodies into milk. This “gut mammary” immunologic axis is an important concept in designing optimal vaccines to provide effective lactogenic immunity [24]. Piglets that regularly suckle the immune mothers are constantly provided with milk-bound IgA antibodies in the lumen. This passive immunity is related to the presence of specific virus neutralizing antibodies in the gastrointestinal tract of suckling pigs. IgG accounts for more than 60% of the immunoglobulins in colostrum. However, IgA is more effective to neutralize the orally infected pathogens than IgG because IgA is more resistant against proteolytic degradation in the intestinal tract and has more virus neutralizing ability than IgG [25]. Accordingly, we carried out IgA-specific ELISA to determine IgA content in the milk and to elucidate the relationship between protection and IgA content in milk of sows and in intestines of piglets. To determine the specific roles of IgA and IgG in preventing PED after vaccination, we investigated the changes in antibody concentrations in milk of sows and in intestines of piglets during lactation. PEDV CHYJ vaccine induced significantly higher PEDV IgA antibody levels in milk and intestines as compared to commercial bi-combined vaccine, which on the other hand induce higher levels of PEDV IgG antibody in milk of sows at earlier stages. However, the two kinds of vaccines did not result in a detectable PEDV IgG antibody response in intestines of all the piglets. These results indicated that PEDV CHYJ vaccine given intranasally was more effective in inducing lactogenic immunity and its transmission via milk in providing passive protection against PEDV than commercial bi-combined vaccine given via i.m. route.

In this study, PEDV-specific IgA and IgG antibody levels were found in milk exhibited from day 0 to day 21 [23], however, their characteristics in mammary secretions have not been studied in detail. It was shown that the levels of total IgA and IgG in milk were significantly higher till d1 post farrowing, which declined slowly later on. Further analysis indicated that IgG levels continued to drop sharply from day 0 to day 21 as compared to IgA levels, the later declined slowly. Moreover, the levels of total IgA in the intestine of piglets were significantly higher at day 1 post farrowing, and declined progressively after that till day 21. Furthermore, there is a strong correlation between the IgA concentration detected in milk of sows and in the intestines of suckling piglets. The overall results indicated that higher IgA antibody levels might play an effective role in neutralizing virus particles especially after farrowing However, achieving uniform lactogenic immunity in herds against PEDV infection still remains a challenge.

During challenge studies, CHYJ intranasal vaccine gives 80% (4/5) protection as compared to commercial i.m. vaccine confering 60% (3/5) immunity in suckling piglets. The protective effects observed in response to high titers of IgA from maternal milk are consistent with the findings of other reports [26]. The variation in protection rate between two vaccines are most probably due to different IgA levels in milk. In fact, the IgA concentration in intestines of piglets before challenge induced in response to intranasal CHYJ vaccine was higher than that in case of commercial vaccine group. The results have proven that IgA plays a more central role in preventing PED infection in piglets than IgG antibodies [27].
PEDV IgA titers in the milk of sows were found strongly correlated with its final titer in the intestine of piglets. More importantly, they were found to be significantly associated with increased protection rate against PED infection. Obviously, improved protective efficiency was mostly dependent on the increase production of IgA in milk of sows and IgA in intestines of piglets. In our study, average value of IgA in milk of sows in CHYJ vaccine group was much higher as compared to commercial vaccine group and vice versa for IgG. IgA memory B cells in swine reside in intestinal (ileum) tissues after gut mucosal immune response [18], so CHYJ vaccine can enhance both PEDV IgA antibody in milk of sows as well as in the intestine of piglets, which increased the correlation between IgA antibody titers and protective efficiency. In this way, our findings have proven that mucosal IgA titers have significant role in providing protection in piglets against PEDV than mucosal IgG titers.

Conclusions

It may be concluded from this study that PEDV CHYJ vaccine can induce lactogenic immunity in sows by intranasal immunization and can protect the suckling neonates against PEDV via colostrum/milk intake.

Abbreviations

PEDV: Porcine epidemic diarrhea virus; TGEV: Transmissible gastroenteritis virus; ELISA: Enzyme-linked immunosorbent assay; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; TCID\textsubscript{50}: Median tissue culture infective dose; RT-qPCR: Real-time quantitative polymerase chain reaction.

Declarations

Acknowledgements

Not Applicable.

Authors’ contributions

GW and AJ designed this study; SD, JW, MM and LZ conducted the experiments; SD and AJ analysed the data; GW and AJ secured the funds; SD prepared the main body of this manuscript; and AJ and MM revised the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

**Ethics approval and consent to participate**

The study was approved by the Laboratory Animal Committee of Guangdong Haid Institute of Animal Husbandry and Veterinary. The experiments were carried out in accordance with the requirements of the Animal Ethics Procedures and Guidelines of the People's Republic of China.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Figures

![Figure 1](image_url)

**Figure 1**

PEDV IgA antibody level in milk of sows. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and all samples tested by PEDV IgA ELISA. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine via intranasal route. Sows in commercial vaccine group were vaccinated with a
commercial bi-combined inactivated vaccine against PEDV and TGEV using intramuscular route. Sows in control group were vaccinated with saline by intranasal immunization.

Figure 2
PEDV IgA antibody in intestines of suckling piglets. The contents of intestines were aseptically collected before eating milk and at day 1, 5, 10, 15 and 21 after offering milk and were tested by PEDV IgA ELISA. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine by intranasal immunization. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route. Sows in control group were vaccinated with saline by intranasal immunization.
Figure 3

PEDV IgA antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before offering milk and at day 1, 5, 10, 15 and 21 after milk intake. All samples were tested by PEDV IgA ELISA kit. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine via intranasal route.
Figure 4

PEDV IgA antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after offering milk. All samples were tested by PEDV IgA ELISA kit. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route.
Figure 5

PEDV IgA antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after feeding milk. All samples were tested by PEDV IgA ELISA kit. Sows in control group were vaccinated with saline by intranasal immunization.
Figure 6

PEDV IgG antibody in milk of sows. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and all samples tested by PEDV IgG ELISA. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine by intranasal immunization. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route. Sows in control group were vaccinated with saline by intranasal immunization.
Figure 7

PEDV IgG antibody in intestines of suckling piglets. The contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after milk feeding and were tested by PEDV IgG ELISA. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine by intranasal immunization. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route. Sows in control group were vaccinated with saline by intranasal immunization.
Figure 8

PEDV IgG antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after feeding milk. All samples were tested by PEDV IgG ELISA. Sows in CHYJ vaccine group were vaccinated with inactivated PEDV CHYJ vaccine by intranasal immunization.
Figure 9

PEDV IgG antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after feeding milk. All samples were tested by PEDV IgG ELISA. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route.
Figure 10

PEDV IgG antibody in milk of sows and in duodenum, jejunum, ileum and colon of piglets. Milk was collected at day 0, 1, 5, 10, 15 and 21 after farrowing and the contents of intestines were aseptically collected before milk intake and at day 1, 5, 10, 15 and 21 after feeding milk. All samples were tested by PEDV IgG ELISA. Sows in control group were vaccinated with saline by intranasal immunization.
Figure 11

The correlation between antibody titers and survival. Sows in CHYJ group were vaccinated with inactivated PEDV CHYJ vaccine by intranasal immunization. Sows in commercial vaccine group were vaccinated with a commercial bi-combined inactivated vaccine against PEDV and TGEV by intramuscular route. Sows in control group were vaccinated with saline by intranasal immunization.