RESEARCH REPORT
SWINE HEALTH

Title: Understanding and searching for immune protection against group C rotavirus in piglets - **NPB # 13-065)**

Investigator: Kyoung-Jin Yoon, DVM, PhD

Institution: Iowa State University

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Industry Summary

Rotavirus is not a new disease within the swine industry; however, there is an evidence of re-emergence and importance in neonates and early weaned piglets. Group C rotaviruses often cause neonatal and pre-weaning diarrhea while group A rotavirus infection is still predominantly a post-weaning enteric disease problem. A typical approach for control of rotavirus-associated disease in piglets is boosting the immunity of dams for the virus and transferring maternal immunity to the piglets until the pig reaches an age at which it is less susceptible to rotavirus infection and associated disease. Commercial and autogenous vaccines have been utilized by producers to control group A rotavirus although those have been variably effective. In contrast, enteric disease associated with group C rotavirus has been more difficult, if not possible, to control because of the inability to grow this rotavirus in laboratory which prevents production of a conventional vaccine. The only effective control measure currently being used is planned exposure (i.e., "feedback") of dams to a farm-specific live rotavirus isolates 2-5 weeks pre-farrow in attempt to boost their immunity against farm-specific virus and enhance transfer of maternal immunity to piglets. Despite the effort, efficacy has been variable and often poor and group C rotavirus continues to be a major problem in neonatal and pre-weaning diarrhea and mortality, raising the need for better understanding of immune-mediated protection against group B or C in piglets.

The proposed study was designed to address if maternal/lactogenic immunity correlates with protection against rotavirus C-associated disease in piglets as well as ontogeny of antibody response to various vaccine forms in old pigs. Two animals experiments were conducted. The first animal experiment involved passive transfer of rotavirus-specific IgG to mimic the maternal immunity in neonates and assess if the level of maternal antibody correlate with protection against oral challenge of rotaviruses. CDCD piglets were fed concentrated IgG raised against a porcine rotavirus C isolate right after birth and monitored for antibody decay and protection against challenge over time. In the second animal experiment, the ontogeny of antibody response of pigs after receiving cell-culture derived live virus, ice cubes containing rotavirus-positive feces and recombinant VP7 was characterize in attempt to estimate the level of material antibody which can be passively transferred to piglets via colostrum. Our study demonstrated that: a) naïve pigs could develop serum antibody response with neutralizing activity when they were given a low dose of live virus orally or injected with a recombinant viral protein; b) virus neutralizing antibody response appeared to be subtype-specific (i.e., G type); and c) orally fed virus-specific immunoglobulin could reduce the severity of disease by rotavirus but not infection.

Our study observations suggest that: a) immunoglobulin feeding can be an option for temporary relief of clinical severity due to group C rotavirus infection and b) while both killed and live vaccines can be options for control, its use depends upon farm status with rotavirus. More importantly subtype matching should be taken into consideration when devising a vaccine for better efficacy.

Keywords: Rotavirus, serogroup C, immune protection, immunoglobulin, vaccination

Scientific Abstract

The proposed study was designed to address if maternal/lactogenic immunity correlates with protection against rotavirus C-associated disease in piglets as well as ontogeny of antibody response to various vaccine forms in old pigs. Two animals studies were conducted for this purpose.

The first animal experiment involved passive transfer of rotavirus-specific IgG to mimic the maternal immunity in neonates and assess if the level of maternal antibody correlate with protection against oral challenge of rotaviruses. CDCD piglets were fed concentrated IgG raised against a porcine rotavirus C isolate right after birth along with milk replacer. Rotavirus-specific antibody was detected by both ELISA and SVN test in sera one day after oral feeding. While ELISA antibody gradually declined over a 4-week period post feeding, SVN antibody disappeared rather quickly between 1 and 2 weeks after feeding. Upon challenge, all pigs developed diarrhea between 24 and 48 hours post inoculation regardless of serum antibody titers. However, the severity of diarrhea, level of virus fecal shedding and degree of villous atrophy were less in IgG receiving pigs than control pigs. Such protective effect against challenge were not apparent after week 2 post feeding. Some degree of heterologous protection was observed in IgG group within 1-week post feeding as compared to control group but was not statistically significant.

In the second animal experiment, the ontogeny of antibody response of pigs after receiving experimental rotavirus vaccines (cell-culture derived live virus, ice cubes containing rotavirus-positive feces, recombinant VP7) was characterize in attempt to estimate the level of material antibody passively transferred to piglets via colostrum. Both live virus and recombinant antigen were able to mount antibody response in naïve 5-week-old pigs which was measurable by ELISA and SVN test. Oral administration of live virus (i.e., feedback) induced lower SVN antibody titers than intramuscular injection of 2-doses of adjuvanted recombinant VP7. Oral administration of live virus, however induced loose stool to mild diarrhea with fecal shedding of the virus in the inoculated pigs. Although VP7 recombinant protein induced a higher SVN antibody titer, such neutralizing activity was subtype-specific and less effective on heterologous strains.

In conclusion, study observations suggest that: a) naïve pigs could develop serum antibody response with neutralizing activity when they were given a low dose of live virus orally or injected with a recombinant viral protein; b) neutralizing antibody response appeared to be subtype-specific (i.e., G type); 3) orally fed IgG could reduce the severity of disease by rotavirus but not infection.

Introduction

Porcine rotavirus (PRoV), discovered in 1970's (Rodger *et al*., 1975), is one of 11 known enteropathogenic viruses associated with clinical diarrhea in swine. Rotaviruses belong to the family *Reoviridae* and have a non-enveloped, double-capsid virion containing 11 double-stranded RNA segments. Structurally, the outer capsids of the virus have three layers: the outer layer (VP7 and VP4), the middle layer (VP6), and the inner layer (VP2) (Chang *et al*., 2012). Rotaviruses can be subdivided into serogroups based on antigenic and sequence differences in the VP6 which is known to be antigenically conserved within each serogroup. To date there are 8 known rotavirus serogroups (A-H) identified (Estes, 2007; Martella *et al.*, 2007); however, only A, B, C and E have been associated with diarrhea in swine. Serogroups A, B and C have been associated with swine diarrhea in the United States, while serogroup E has only been identified in the United Kingdom (Chang *et al*., 2012). Each serogroup can be further classified into subtype/serotype based on antigenic and genetic differences in VP4 (P type) and VP7 (G type). Cross protection of pigs infected with different serogroups of rotavirus is poor; partial protection among different subtypes within a serogroup has been reported.

It is widely accepted that infection is associated with moderate economic impact in the swine industry (Holtkamp *et al*., 2007). Infection with multiple serogroups is not uncommon in swine (Will et al., 1974). Furthermore, multiple or sequential infections with rotaviruses of the same serogroup may occur as they may be antigenically distinct in the outer capsid layer (Paul *et al*., 1993; Winiarczyk *et al*., 2002). In short, almost all pigs are exposed to multiple different rotavirus strains and serogroups throughout the neonatal and post-weaning phase of production. Seroprevalance of antibody against both serogroup A and C rotaviruses approaches 100% of pigs as they reach market weight (Chang *et al*., 2012).

Clinical signs in piglets are characterized by watery to creamy feces which are often yellow or white in color. Diarrhea is due to enterocyte destruction and sloughing with contraction of villus cores by rotavirus invasion leading to decreased absorptive capacity of intestine (Chang *et al*., 2012). The incubation period is 18 to 96 hours and is followed by depression, diarrhea, and sometimes fever. If no other complications or concurrent infections are present, the diarrhea ceases within 2 or 3 days. However, neonatal rotavirus type C (or A) infections are often complicated by *Clostridium difficile* infections in the first few days of life and post-weaning rotavirus type A (or C) infections by F18+ or K88+ hemolytic *E. coli*. Mortality (5-15%) and morbidity (20-80%) associated with rotavirus infection vary depending on factors such as virus strain, pig immunity, concurrent infections, and environmental conditions (Saif, 1999). Serogroups A and C are known to be associated with more severe diarrhea and microscopic lesions as compared to serogroup B rotavirus.

Although rotavirus infection can affect pigs of any age, it is most often detected in piglets less than 8 weeks old. According to diagnostic data of Iowa State University VDL diagnostic data (Madson *et al*., 2012) and MN VDL data (Torrison *et al*., 2010), detection of serogroup C rotavirus is much more common (56%) in pre-weaned pigs (<7 days of age) while group A rotavirus was detected more commonly (51%) in post-weaned pigs (21-42 days of age). Maternal antibodies persist for 3-6 weeks. In general, rotavirus-associated diarrhea occurs when the viral exposure dose exceeds the protective immunity of dams (Saif, 1985) and is also dependent on pig age with older pigs becoming resistant.

There is no specific treatment other than supportive therapy for rotaviruses. The use of drinking water containing electrolytes and glucose improves clinical situation including mortality rate (Paul and Stevenson, 1999). In addition, the use of isotonic intraperitoneal injection can be useful, particularly in farms with a low number of affected litters. Proper environmental conditions and pig handling help to minimize losses. Commercial and autogenous vaccines have been utilized by producers and have been variably effective in control of group A rotavirus. Enteric disease associated with rotavirus groups B or C has been often more difficult, if not possible, to control because of the inability to grow these rotaviruses in laboratory (Bridger, 1994; Sanekta et al., 1996) which prevents production of a vaccine. In the field, feedback or live virus inoculation (LVI) has been practiced on sows for control of rotavirus B and C associated enteritis in piglets. Sows are often given fecal material confirmed to be PCR positive for rotavirus group B or C approximately 2-5 weeks before their expected farrowing date in an attempt to boost their immunity and enhance transfer of

maternal immunity to the piglets until the pig reaches an age at which it is less susceptible to rotavirus infection. Efficacy has been variable. The approach has not been validated or optimized under experimental condition. Therefore, clear understanding of immune-mediated protection for rotavirus B or C in piglets is currently lacking.

Objectives

1. To determine if passively transferred anti-porcine rotavirus C immunoglobulin provides piglets protection against group C rotavirus

2. To determine if pigs develop protective immune response against group C porcine rotavirus by vaccination with various forms of the virus

Materials & Methods

Objective 1

A total of 72 caesarian-derived, colostrum-deprived (CDCD) neonatal pigs were randomly divided into two groups and fed with 2ml of either group C RoV-specific immunoglobulin G (IgG) or normal serum globulin (NSG) preparation along a commercial colostrum replacer with 2 hours after birth. IgG and NSG were prepared in rotavirus-negative 5-week-old pigs inoculated with a cell-culture derived group C PRoV isolate 25060 (G6) or sham inoculum. The antibody level in each pig serum was assessed at 24 hours after feeding using VP6 and VP7 peptide-based ELISAs and serum-virus neutralization (SVN) assay. Then decay of passively transferred antibody in blood circulation was monitored by bleeding all pigs at each challenge day.

Eight pigs each from IgG and NSG groups were randomly selected, moved to one of 6 (or 3) new rooms, and challenged orally with 10^2 $TCID_{50}$/pig of homologous PRoV C (25060) or heterologous PRoV C (24122, G5) or sham control (i.e., virus-free media) at day 1 and thereafter weekly (1 to 5) post feeding. Antibody-mediated immune protection was assessed by monitoring the presence/absence of clinical sign (diarrhea) and virus shedding in fecal swabs for 48 hours post inoculation (PI). At 48 hours PI, pigs were euthanized and necropsied for histopathology (intestine) and virus assay (intestinal content).

Objective 2

Experimental vaccines for the PRoV C 25050 were prepared in the following formats: a) cell culture derived whole virus (LV); b) ice cube containing semi-purified feces/content positive for PRoV C which was collected from objective 1 study (FIC); and c) VP7 recombinant proteins with oil adjuvant (KV). LV and FIC were adjusted to 10^3 $TCID_{50}$/ml and KV was prepared to contain 0.3mg of VP7 per 1ml. Each vaccine preparation was given via oral (LV, FIC) or intramuscular routes (KV) to rotavirus-negative 5-week-old pigs grown from CDCD piglets in an isolation room. While LV and FIC was given once, KV was given twice with a 2-week interval. Ten pigs were assigned to each treatment group. One group of 10 pigs served as sham control group and received media only.

Pigs were monitored daily for development of diarrhea or any noticeable clinical signs (i.e., elevating body temperature, lethargy, appetite, vomiting, diarrhea) for first 7 days after vaccination. Fecal swabs were collected from all pigs on day 0, 3, 7, 10, 14, 21, 28 and 35 post vaccination and tested for shedding of rotavirus C by PCR. Pigs were bled on the day of vaccination and every 7 days until 5 weeks after vaccination. Antibody response of pigs to vaccination was assessed using VP6 and VP7 peptide-based ELISAs and SVN assay.

Results

Objective 1

No rotavirus-specific antibody (VP6, VP7 and SVN) was detectable in any of sera collected from piglets fed NGS at any of challenge days. Consequently, all pigs became diarrheic after challenge between 24 and 48 hours PI regardless of virus strain given (i.e. 25060 or 24122). Fecal shedding of rotavirus was also detected during 48 hours PI; however, level of virus shedding was lower in older pigs (4-5 weeks of age) than younger pigs. By sequencing for VP7, no cross contamination between treatment groups occurred. Microscopically, villous atrophy was apparent in all groups at 48 hours PI. Degree of atrophy was significantly milder in pigs challenged at 5 weeks post feeding than other pigs.

In pigs received group C PRoV (G6)-specific IgG at birth, VP6 and VP7-specific ELISA antibodies were detected in sera collected at one day after feeding. The day 1 sera also showed neutralizing activity (1:16) against PRoV C 25060 (G6, homologous strain) but not against PRoV C 24122 (G5, heterologous strain). SVN activity disappeared quickly between week 1 and 2 post feeding whereas VP6 and VP7-specific ELISA antibody gradually declined and continued to be detectable in pig sera until week 4 and 3 post feeding, respectively. Upon challenge all pigs developed diarrhea between 24 and 48 hours PI regardless of serum antibody titers. However, the severity of diarrhea, level of virus fecal shedding and degree of villous atrophy varied among treatments. In comparison to NSG group, all IgG groups showed less severe diarrhea when challenged with the homologous virus based on fecal scoring. More importantly, level of virus shedding in feces and degree of villous atrophy were significantly less in IgG receiving group than NSG group. Such protective effect against challenge were not apparent after week 2 post feeding, except age-dependent clinical response to the infection. Some degree of heterologous protection was observed in IgG group within 1-week post feeding as compared to control group but was not statistically significant. Pigs had less severe diarrhea and villous blunting and lower level of virus in feces when challenged with the homologous virus as compared to ones challenged with heterologous strain, particularly day 1 and week 1 post feeding.

Objective 2

Both LV and FIC were able to mount antibody response in pigs between 7-14 days post inoculation. VP6 ELISA antibody (IgG) was detectable in some pigs at day 7 post inoculation while VP7-specific antibody was apparent after 2 weeks post inoculation. However, ELISA antibody peaked at 3 weeks post inoculation and started to decline. SVN antibody against the PRoV C 25050 (homologous strain) was also detectable at a low level (1:2-1:4) after 2 weeks post inoculation and reached to 1:16-1:32 by 35 days post inoculation. 35 DPI sera show weak SVN activity (1:2-1:8) against PRoV C 24122 (heterologous strain). Pigs were lethargic and developed loose stool to mild diarrhea after receiving LV and FIC, lasting 2-3 days. Rotavirus was detectable in their feces during the first week of inoculation. No elevating temperature was apparent in any of these pigs.

In pigs injected with KV, VP7-specific antibody (IgG) was detectable after 2 doses and continue to present until the end of study. No VP6-specific antibody was detected throughout the study. A good level of SVN antibody (1:32-1:128) against PRoV C 25050 was detectable at 35 days post injection. However, no SVN activity against PRoV C 24122 was observed. None of the pigs developed diarrhea and shed rotavirus in feces.

Discussion

Two animal studies were conducted to address if maternal/lactogenic immunity correlates with protection against rotavirus C-associated disease in piglets as well as ontogeny of antibody response to various vaccine forms in old pigs. In summary, the studies showed that: 1) naïve pigs could develop serum antibody response with neutralizing activity when they were given a low dose of live virus orally or injected with a recombinant viral protein; 2) neutralizing antibody response appeared to be subtype-specific (i.e., G type) under study conditions;

and 3) orally fed IgG could reduce the severity of disease by rotavirus but not infection. These observations suggest a few implications. First, subtype matching (i.e., G and/or P type) should be taken into consideration when devising a vaccine for better efficacy. For this, continuous monitoring of predominant subtype(s) in pig population in the US may be necessary. Second, virus-specific immunoglobulin (e.g., egg yolk antibody) may provide temporary relief of clinical severity against challenge when orally given; however, such a treatment should be used with knowledge that the efficacy can be limited and may not be long. Third, sufficient time should be given to sows if feedback is used as a way to stimulate a high level of lactogenic antibody in the sows.

This project did not address whether or not vaccination and which vaccine type can provide adequate protection since no challenge was involved. Based on knowledge and experience with other enteric viral pathogens such as TGE virus or PED virus, an inactivated virus vaccines have a merit in rotavirus-endemic herds while live virus vaccines may be more effective on pigs in naïve herds. Nonetheless other factors (e.g., delivery system, dose, route, etc) can impact the efficacy of vaccines.

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