EVALUATION OF TRANSMISSIBLE GASTROENTERITIS AND PORCINE RESPIRATORY CORONAVIRUS IMMUNOLOGIC INTERACTION IN SERONEGATIVE PREGNANT GILTS

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The primary objective of this study was to determine if Porcine Respiratory Coronavirus (PRCV) sensitized gilts responded to intramuscular (IM) vaccination with Transmissible Gastroenteritis Virus (TGEV) in a similar fashion as gilts sensitized to TGEV alone. It was demonstrated that pregnant gilts sensitized to PRCV, prior to TGEV vaccination, do not produce significantly higher TGEV serum neutralizing antibody titers when compared with gilts sensitized to either PRCV or TGEV alone. Anti-TGEV IgG and IgA levels were assayed for in post-farrow milks from all gilt groups. Only IgG was detected which indicated a lack of mucosal priming. The second objective of this study was to determine if animals, which have recovered from a TGEV infection, can be infected with PRCV. TGEV antibodies circulating in these exposed piglets prevented PRCV infection as demonstrated by the absence of PRCV shedding during the post-challenge period. It was found that these piglets had an eight-fold boost in TGEV serum neutralizing antibody titers 21- days post PRCV challenge. Piglets that had not been exposed to TGEV significantly shed virus during this same post-challenge period and exhibited a three-fold boost in TGEV neutralizing antibody titer. Piglets that had suckled TGEV immune dams were also, for the most part, protected from PRCV shedding but exhibited no boost in TGEV serum neutralizing antibody titer.

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INTRODUCTION AND REVIEW OF LITERATURE

Transmissible Gastroenteritis Virus (TGEV) is a coronavirus that infects swine of all ages. It replicates in the cytoplasm of absorptive epithelial cells located in the villi of the small intestine (14). Virus infection occurs orally and the virus is shed in the feces and in respiratory secretions of infected animals. TGEV appears in two clinical forms: epizootic and enzootic (8, 9). In its epizootic form, TGEV replication results in a degeneration or shortening of the small intestinal villi decreasing its absorptive capability. This villus atrophy results in severe watery diarrhea which causes piglets to die of dehydration during the first two weeks of age. In the enzootic, or chronic form, persistent infection of a herd with TGEV is a direct result of continual or frequent introduction of susceptible animals into the immune or partially immune herd. Therefore, the disease is perpetuated from infected animals to susceptible animals (9, 19). TGEV diarrhea in its chronic form usually occurs in piglets older than two weeks of age and can persist through two weeks post-weaning. TGEV is considered the prototypical enteropathogenic virus and is often used in models to study pathophysiology and pathogenesis of viral diarrhea(14).

Unlike humans, pigs cannot transfer immunoglobulins (Ig) transplacentally. Therefore, at birth piglets are devoid of Igs which are then subsequently obtained by suckling milk from an immunized dam. Passive immunity is achieved by ingestion of milk antibody providing the neonate with the same complement of antibodies as the dam (18). This ability to ingest high levels of colostral Ig is essential to the piglet's survival. There are three classes of Igs found in sows milk: IgG, IgM and IgA. IgG is the most abundant Ig in the colostrum accounting for 80% of the total Ig content (16). Within the first week of lactation, the concentration of IgG decreases 30-fold (18). IgM accounts for 5% of the total Ig in colostrum which makes it the least abundant of the three classes (16). After three days of lactation, IgM is usually undetectable (19). IgA antibodies (Abs) provide optimal passive immunity to TGEV because concentrations decline only 3-fold during the first week of lactation becoming the predominant milk Ig and providing the most effective protection. Reasons why IgA has greater efficacy than the other two Igs include 1). they are found at higher levels than the other Igs, 2). they are resistant to intestinal proteolytic enzymes, and 3).they bind to the gut enterocytes. Even though piglets do not absorb secretory IgA, its presence is still important in passive immunity. Pensaert and others have reported that pigs which have recovered from TGEV infection can transfer passive protection to their suckling

piglets by the frequent ingestion of colostrum and milk that contain TGEV-neutralizing Abs. These Abs neutralize the virus in the lumen of the intestine preventing the virus from infecting susceptible epithelial cells. Haelderman termed this process "lactogenic immunity". Since secretory IgA is produced in mammary tissue by cells seeded from the intestine, this "gut-mammary" association is important in designing vaccination programs in which lactogenic immunity is utilized (18).

Since IgA is produced by a lactating sow as a consequence of an intestinal infection, it is advantageous that pigs be immunized orally as well as intramuscularly (IM). When pigs are immunized IM only, a circulatory IgG antibody response is produced which provides most of its protection systemically, not intestinally. Although a lactating sow immunized IM may offer some protection to her piglets during the first week of life, it is not lasting due to at least a ten-fold drop of IgG antibody 48 hours after farrowing (20). This results in IM immunization not being the most effective route for protection of suckling piglets in that there is little or no gut immunity to TGEV during lactation. But if the sow is immunized orally, the gut's active immunity stimulates IgA lymphocytes (4). These IgA producing lymphocytes migrate to the mammary gland resulting in local IgA production (11). In lactating sows, this IgA is longer-lasting (decreasing 3-fold vs IgG's 30-fold in one week) and protective when it is continually ingested by the piglets. Intramuscular inoculation with TGEV (virulent or attenuated) during the gestation of swine, who have been previously primed orally with TGEV, result in a boost in milk Ab's of both IgG and IgA classes. Therefore, if a sow is vaccinated IM only, her piglets do not absorb the longer lasting, protective milk IgA and become sick when infected with TGEV. This is because TGEV antibodies (IgA) are at a low level leaving only IgG antibodies, which do not provide optimal protection (19). With these things in mind, the best vaccination program for a pregnant dam must involve both an oral route, to stimulate the production of IgA, and an IM route, to stimulate IgG and boost IgA production. Without oral stimulation, a less effective antibody response is produced.

Ambico, Inc. has developed an oral vaccine against TGEV which takes into account the biology of Igs mentioned above. The vaccine is administered both orally and intramuscularly assuring that higher and more persisting TGEV milk antibody levels are produced. This is accomplished by the vaccine virus multiplying in the sow's oral-pharyngeal cavity at which point the primed lymphocytes migrate towards the Peyer's patches. Here, in the ileum of the small intestine, the lymphocytes continue to differentiate and stimulate the production of IgA lymphocytes. These lymphocytes migrate to the

supramammary lymph nodes and secrete IgA antibodies into the milk which allows enteric protection of the suckling piglets from virulent TGEV challenge (20). Therefore, this vaccine protects piglets via passive immunization rather than active immunization in that active immunity involves the development of humoral immunity in response to stimulation by antigen (10).

A TGEV serological survey was done in Belgium among a sow population. Sixty eight percent of these animals exhibited an unexpectedly high incidence of antibodies against TGEV even though an increase incidence of diarrhea was not seen. No TGEV vaccination is performed in Belgium, so this finding could not be explained and indicated that a non-enteropathogenic virus related to TGEV had appeared. Following this discovery, the virus was isolated in both pigs and cell culture. Further studies indicated that this virus replicates in high titers in the tonsilar tissues and the respiratory tract. Replication has also been found to occur in nasal, tracheal, bronchial, bronchiolar and alveolar epithelial cells and in alveolar macrophages (1). This virus was subsequently named Porcine Respiratory Coronavirus (PRCV). Despite all of the areas of replication, no enteric infection exists and even the respiratory tract infection is usually asymptomatic (3, 12, 13, 23). Therefore, PRCV is spread through herds aerogenically. The behavior of TGEV and PRCV, even though they produce different pathology, are closely related antigenically. Porcine Respiratory Coronavirus induces antibody responses in pigs that cannot be distinguished from TGEV infection via standard serological assays (3). In 1990, Rasschaert et al. found that PRCV contained a 224 amino acid deletion in the S, or spike, glycoprotein which was not seen in TGEV (17). The S glycoprotein is one of three structural proteins (the others are membrane and nucleoprotein) of TGEV and PRCV and is responsible for the induction of neutralizing antibodies (7). Using these antigenic differences, specific monoclonal antibodies have been developed to distinguish between these two viruses and their antibodies (3).

Porcine Respiratory Coronavirus has also been detected in the United States and was first isolated by Dr. Howard Hill in 1989 at the Veterinary Medical Research Institute in Ames, Iowa. This isolate was obtained from a pig nasal swab sent to Dr. Hill from an Indiana herd. The virus was propagated in swine testicular cells and designated ISU-1 (1). Using the PRCV-TGEV differentiating monoclonal antibodies mentioned previously, this PRCV isolate has been found to be similar, but not identical, to those found in Europe. Further studies have indicated a 227 amino acid deletion in the S glycoprotein which differs from the 224 amino acid deletion seen in European PRCV (22).

A decrease was seen in clinical TGEV infections in Europe at approximately the same time that seroconversion (4-fold increase in serum neutralizing antibody titers) to PRCV was detected in swine and has led researchers to speculate whether pregnant animals that have been sensitized to PRCV will protect their piglets from TGEV infections (13). Studies done at Ambico, Inc. have determined that piglets inoculated orally and intranasally with PRCV, followed by an oral TGEV challenge 14-days later, developed watery diarrhea within 48 hours post-challenge. This reaction mimicked symptoms seen in non-inoculated controls challenged at the same time. Morbidity rates for all animals, including non-inoculated controls, ranged from 42 to 50% while piglets vaccinated orally with TGEV remained clinically normal post-challenge. This study indicated that piglets previously exposed to PRCV will not be protected from virulent TGEV. A similar study has been published using the European PRCV confirming the above results (13). Antigen sensitization at the respiratory level results in the production of IgG as the predominant Ig class, therefore, protection on the enteric level is not lasting (13). If piglets that have suckled PRCV positive sows were challenged with virulent TGEV at three days of age, they would be protected since there are still protective IgG antibodies in the piglet's gut. But if the piglets were challenged at seven days of age, they would not be protected due to the rapid decrease in IgG by that time. This is another indication that PRCV is not a good candidate for a vaccine against TGEV because it does not elicit long lasting IgA antibodies.

Nonetheless, previously published reports have indicated that PRCV can induce the secretion of IgA antibodies in milk and that these antibodies have the capacity to neutralize TGEV (15). Other studies report that natural infection with PRCV induces protective lactogenic immunity against TGEV(2, 6). These studies, as well as others, have continued the speculation as to whether PRCV- infected gilts can passively protect their piglets from TGEV infection. This research will determine whether gilts sensitized with PRCV will respond to vaccination with TGEV in a similar fashion to that of sensitized gilts. IgG and IgA levels will be determined from colostra, seven, and fourteen day post-farrow milks. The second objective will determine if animals that have recovered from a TGEV infection can be subsequently infected with PRCV.

MATERIALS AND METHODS

Animals

Fourteen pregnant gilts were purchased from H + K Enterprises of Ames, Iowa, and were delivered nine weeks pre-farrow. These gilts were screened for anti-TGEV antibodies and all were sero negative. The PRCV exposed gilts were kept separate from the other animals in the study and strict isolation procedures were applied to avoid aerosol spread of virus.

Viral inocula

Porcine Respiratory Coronavirus ISU-1/ST-7 virus isolate was obtained from Dr. Howard Hill of the Veterinary Medicine Research Institute in Ames, Iowa. It was passed in swine testicular (ST) cells seven times and its titer was determined to be $10^{9.0}$ $TCID_{50}$/ml.

The TGEV vaccine used was Ambico's Prosystem 1 serial #614 (Ambico, Inc. Dallas Center, Iowa) which had a titer of $10^{7.3}$ $TCID_{50}$/ml.

Virulent TGEV Standard Challenge (SC) lot-4 was prepared by infecting gnotobiotic piglets with virulent TGEV. Upon the first signs of clinical infection, the piglets were sacrificed and the small intestinal contents collected as a 50% extract. The contents are free of pathogenic bacteria. By titrating the isolate in susceptible piglets, a Pig Infectious Dose level was determined. In this study, 1000 Pig Infectious Doses (PID's) per baby pig were used for challenge.

Vaccination of Gilts

Eight of the fourteen gilts (designated groups A and B) were inoculated with PRCV at eight weeks pre-farrow which allowed them sufficient time to recover from infection. The dosage was given 1 ml orally in a corn-milk mash and 2 ml given intranasally using an atomizer in order to split the dose equally between nares during inspiration.

Four of the PRCV infected gilts (group A) and two others not infected with PRCV (group C) were given an IM vaccination with TGEV-Rotavirus vaccine at five and two-

weeks pre-farrow. The vaccine used was a single dose with each individual vial being reconstituted with 2 ml of sterile diluent prior to use.

The remaining four gilts served as non-vaccinated controls (designated groups D and E).

Refer to Table 1 for a summary of gilt vaccinations.

Gilt Sample Collection and Testing Parameters

All gilts were bled at time of arrival, five-weeks pre-farrow, day of farrow, fourteen days post-farrow, and twenty-eight days post-farrow. Blood samples were allowed to clot, centrifuged at 1000 x g for 20 min, and the sera collected. The sera was then inactivated for 30 min at 56° C and assayed for TGEV serum neutralizing antibodies using a varying serum-constant virus assay. Fifty to five hundred $TCID_{50}$'s of indicator virus were used to determine anti-TGEV titers. In a 96-well microtiter plate, serum dilutions were incubated with the amount of indicator virus mentioned above, at 25° C for 2 hours. Following incubation, 100,000 ST cells per well were added to the serum-virus mixture. The plates were incubated at 37° C for three days. The serum neutralization titers were calculated using a Spearman-Karber 50% Endpoint Table.

Colostra and milks were collected at time of farrow, seven days post-farrow, and fourteen days post-farrow. The samples were centrifuged and the milk collected below the top layer of fat. Following heat inactivation for 30 min at 56° C, the colostra and milk were assayed for TGEV serum neutralizing antibodies as previously described.

After the colostrum and milk neutralization titers were calculated using the Spearman-Karber 50% Endpoint Table, they were assayed for IgG and IgA immunoglobulin content against TGEV. This was done using an indirect ELISA system in which 96-well Immulon - 2 ELISA plates were coated with TGEV antigen and ST cell lysate. The antigen was propagated in tissue culture, cell debris removed via low speed centrifugation, and concentrated 100x via high speed centrifugation. The cell lysate was prepared by first removing the cells from a confluent roller bottle by freezing and thawing. The cell debris was removed via low speed centrifugation and the supernatent collected. This was concentrated 100 x via high speed centrifugation. Every other row of wells were coated with a predetermined dilution of TGEV antigen while the rest of the wells were coated with the same dilution of cell lysate. The plates were incubated at 4°C overnight. After blocking for two hours at room temperature using PBS/0.05% Tween 20/1% bovine

serum albumin, test colostrum and milk samples were added, diluted as desired, and incubated at 25° C. After washing the plates, a solution of IgG and/or IgA anti-swine antibodies conjugated with horse radish peroxidase was added to detect IgG and IgA levels. Following incubation and washing, a 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate](ABTS) substrate solution was added and allowed to develop. Plates were read at 405 nm on an ELISA plate reader. Calculations were done by subtracting the readings obtained on the cell lysate dilutions from those obtained on the corresponding, virus-concentrate dilutions. This gave the ELISA specific reaction value. A value of 0.2 and higher was considered positive.

Clinical Observations were made daily and litter score sheets were presented detailing the number of animals born alive and dead. Also, feces were scored as normal, creamy or watery.

TGEV and PRCV Piglet Challenges

Baby pigs in groups B, C and D were orally challenged with $10^{3.0}$ PID's of virulent TGEV standard challenge lot-4 at fourteen days of age.

At time of weaning (28 days of age), piglets in groups A, D and E, were challenged 1.0 ml orally and 1.0 ml intranasally (0.5 ml/nare) with PRCV.

Refer to Table 1 for a summary of piglet challenges.

Piglet Sample Collection and Testing Parameters Post PRCV Challenge

Blood samples were collected at time of challenge and 21 days post-challenge. They were processed as before and assayed for serum neutralizing antibodies to TGEV as described previously. Geometric mean titers of all groups were calculated by taking the log of each sample titer, averaging them, and then taking the antilog.

Throat and nasal swabs were collected starting at five days post PRCV challenge and continuing through fifteen days post PRCV challenge. One swab was used for both the nose and throat. Following swabbing, the swab was placed in a test tube containing 1.5 ml RMM with 200 µg/ml gentamicin. The combination was centrifuged at 1000 x g for 20 min. The liquid was expressed from the swabs on the side of the collection tube and an equal volume of 50% sucrose was added giving a final sucrose concentration of 25%. Swab samples were assayed for PRCV by plaque assay. This was done by infecting

confluent ST 6-well microtiter plates with serially diluted swab samples. A Noble agar overlay was added to each well and incubated at 37°C for three days. Following the incubation period, another Noble agar overlay with neutral red dye was added to each well and incubated at 37°C overnight. Plaques that were 1 mm in diameter were observed and counted as positive (for presence of plaques) or negative (for absence of plaques).

Refer to Table 1 for a compete summary of gilt and/or piglet vaccination, challenges, and data collection parameters performed in this study.

Table 1. Gilt vaccination, piglet challenge and sample collection schedule

	Gilt treatment schedule			Piglet treatment schedule		
Day of Test	Groups	Bleed	Inoculate/Vaccinate	Milk	Challenge	Throat and nasal swabs
9-WPF[1]	A, B, C, D, E	X				
8-WPF	A, B		X(PRCV;Oral and IN[5])			
5-WPF	A, B, C, D, E	X				
	A, C		X(TGEV;IM[6])			
2-WPF	A, C		X(TGEV;IM)			
DOF[2]	A, B, C, D, E	X		X		
7-DPF[3]	A, B, C, D, E			X		
14-DPF	A, B, C, D, E	X		X		
	B, C, D				X(TGEV:oral)	
28-DPF	A, B, C, D, E	X				
	A, D, E	X			X(PRCV;Oral+IN)	
5 through 15-DPW[4] (Grps A, D, E)						X

1. WPF = Weeks Pre-Farrow
2. DOF = Day of Farrow
3. DPF = Days Post-Farrow
4. DPW = Days Post-Weaning
5. IN = Intranasal
6. IM = Intramuscular

Statistical analysis

Student's T test was used to compare data from between sow groups and piglet groups.

RESULTS

Effect of Sow Inoculations

All sows had normal farrowings except for two. One sow had only one piglet which died two days later while the other sow killed three of its four piglets by not allowing them to suckle. None of the inoculated sows exhibited any signs of illness post PRCV inoculations or TGEV vaccinations. One sow from the non-vaccinated control group refused to eat for five days following TGEV challenge of piglets while one gilt from the PRCV group refused to eat for one day and developed a soft stool for one day following piglet challenge. This lack of appetite, from these sows, was thought to be an indication of TGEV infection.

All TGEV neutralizing antibody titers for pre-vaccination sera, day of farrow sera and colostra, seven day post-farrow milks, fourteen day post-farrow sera and milks and twenty-eight day post-farrow sera, are shown in Table 2. All inoculated/vaccinated sows seroconverted (four-fold increase in antibody titer) to TGEV at the time of farrowing while the non-vaccinated controls remained seronegative for TGEV. All virus exposed groups had statistically significant ($P < 0.05$) colostral TGEV antibody titers compared with the non-vaccinated control group. All sera and milk titers decreased over time except for a dramatic jump in anti-TGEV titers seen in 28 day post-farrow sera of non-vaccinated, PRCV inoculated, and TGEV vaccinated sows. Piglets from these three gilt groups were challenged with TGEV at 14 days of age and subsequent virus shedding may have boosted the sows antibody titers. These boosts in antibody titers indicate an infection took place and that the inoculations/vaccinations were not protective. This is further demonstrated in that TGEV vaccinated and PRCV inoculated sows exhibited a statistically significant ($P < 0.05$) increase in antibody titer compared to the non-vaccinated gilts.

Table 2. Sow neutralizing antibody titers vs TGEV

Animal #	Inoculation group	Pre vaccination sera	Day of farrow Sera	Day of farrow Colostrum	7 DPF[1] Milk	14 DPF Sera	14 DPF Milk	28 DPF Sera
78	A[2]	≤2.0	57	299	2.0	21	2	NS[3]
79		2.8	197	452	86	NS	NS	NS
84		≤2.0	49	260	11.3	22.7	5.7	NS
87		≤2.0	86	393	22.7	16	≤2.0	11.3
82	B[4]	4.0	113	197	28	21	16	≥3620
85		≤2.0	33	171	≤2.0	32	≤2.0	1370
86		4.0	86	171	NS	33	28	3140
88		4.0	19	149	NS	NS	NS	NS
77	C[5]	≤2.0	299	299	16	33	5.7	2080
93		≤2.0	171	197	33	32	2.8	904
90	D+E[6]	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	37
92		≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	22.7
89		≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	NS
138		≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0

1. DPF = Days Post Farrow
2. Group A Vaccination: PRCV - $10^{9.5}$ $TCID_{50}$/ml; 1 ml oral + 2 ml IN(8-WPF)
   TGEV- $10^{7.6}$ $TCID_{50}$/ml; 2 ml IM (5 + 2-WPF)
3. NS = No Sample
4. Group B Vaccination: PRCV - $10^{9.5}$ $TCID_{50}$/ml; 1 ml oral + 2 ml IN(8-WPF)
5. Group C Vaccination: TGEV- $10^{7.6}$ $TCID_{50}$/ml; 2 ml IM (5 + 2-WPF)
6. Group D + E Vaccination: Non-Vaccinated Controls

All of the sow colostrum and milk samples were evaluated for IgG and IgA type immunoglobulins. Table 3 shows that all inoculations (TGEV, TGEV/PRCV, and PRCV) stimulated IgG responses, which significantly decreased by 7 days post-farrow. The strongest IgG responses were seen in the PRCV/TGEV and TGEV only groups. No detectable IgA responses were seen in any colostra or milk samples.

Table 3. IgG and IgA TGEV neutralizing antibody levels in sow colostra and milks

Animal #	Inoculation group	DOF[1] Colostrum IgG	DOF[1] Colostrum IgA	7 DPF[2] Milk IgG	7 DPF[2] Milk IgA	14 DPF Milk IgG	14 DPF Milk IgA
78	A[3]	3200	≤10	≤10	≤10	≤10	≤10
79		2000	≤10	≤10	≤10	NS[4]	NS
84		1000	≤10	160	≤10	≤10	≤10
87		16,000	≤10	≤10	≤10	≤10	≤10
82	B[5]	1600	≤10	≤10	≤10	≤10	≤10
85		800	≤10	≤10	≤10	≤10	≤10
86		3200	≤10	NS	NS	≤10	≤10
88		≤10	≤10	NS	NS	NS	NS
77	C[6]	16,000	≤10	80	≤10	≤10	≤10
93		16,000	≤10	320	≤10	80	≤10
90	D+E[7]	≤10	≤10	≤10	≤10	≤10	≤10
92		≤10	≤10	≤10	≤10	≤10	≤10
89		≤10	≤10	≤10	≤10	≤10	≤10
138		≤10	≤10	≤10	≤10	≤10	≤10

1. DOF = Day of Farrow
2. DPF = Days Post-Farrow
3. Group A Vaccination: PRCV - $10^{9.5}$ $TCID_{50}$/ml; 1 ml oral + 2 ml IN(8-WPF)
   TGEV- $10^{7.6}$ $TCID_{50}$/ml; 2 ml IM (5 + 2-WPF)
4. NS = No Sample
5. Group B Vaccination: PRCV - $10^{9.5}$ $TCID_{50}$/ml; 1 ml oral + 2 ml IN(8-WPF)
6. Group C Vaccination: TGEV- $10^{7.6}$ $TCID_{50}$/ml; 2 ml IM (5 + 2-WPF)
7. Group D + E Vaccination: Non-Vaccinated Controls

Effect of TGEV and/or PRCV Challenge on Piglets

All the piglets from sow groups B, C, and D were challenged at 14 days of age with TGEV. All piglets exhibited creamy to watery diarrhea that persisted four to seven days. Piglets from group C gained nearly twice the weight of the group B piglets while group D piglets lost weight (data not shown).

All piglets from sow groups A, D, and E were challenged at 28 days of age with PRCV. None of the piglets exhibited clinical respiratory signs at any time during the 21-day post-challenge period. Piglets from groups A gilts did not significantly shed PRCV post-challenge. Piglets from group D gilts, also challenged at 14 days of age with TGEV, did not shed PRCV during the post-challenge period. Thirteen out of seventeen piglets from group E gilts shed PRCV 7 days post-challenge, decreasing significantly 8 days post-challenge. See Table 4 for data.

Table 4. PRCV Isolation from nasal and throat swabs

Inoculation of gilts	Inoculation(s) of piglets	Virus shedding post PRCV challenge nasal and throat swab isolations 7-DPC[1]	8-DPC	9-DPC
Uninoculated	TGEV(O)/PRCV(IN-O)[2]	0/16	0/16	0/16
PRCV(O-IN)/TGEV(IM)	PRCV(IN-O)[3]	0/21	2/21	0/21
Uninoculated	PRCV(IN-O)[3]	13/17	1/17	0/17

1. DPC = Days Post-Challenge

2. Piglets were challenged 2 ml orally with $10^{3.0}$ PID_{50} of TGEV at 14 days post farrow with 1 ml oral and 1 ml intranasally with $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days post-farrow.

3.Pigets were challenged 1 ml oral and 1 ml intranasally with PRCV $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days post-farrow.

Day of PRCV challenge and 21-day post-challenge sera were assayed for neutralizing TGEV antibodies for all the previously mentioned piglets. All PRCV challenged piglets that suckled group A sows exhibited no increase in anti-TGEV titers. PRCV challenged piglets that suckled group D sows, and were challenged 14 days of age with TGEV, exhibited an eight-fold average increase in anti-TGEV titers by 21 days post PRCV challenge. Piglets that suckled group E sows, exhibited a three-fold average increase in anti-TGEV titers by 21-days post PRCV challenge. All data are shown in Table 5.

Table 5 . Piglet serum neutralizing antibodies vs TGEV

	Inoculations		Piglet serum virus titer	
Animal #	Gilts	Piglets	Day of PRCV Challenge	21 DPC[1]
4-1	Uninoculated	TGEV(O)/PRCV(O-IN)[2]	43	227
4-2			33	33
4-3			28	260
4-4			≤2.0	130
4-5			11.3	86
4-6			43	299
4-7			43	343
4-8			32	149
12-1			≤2.0	149
12-2			32	227
12-3			49	NS[3]
12-4			25	98
12-5			22.7	NS
12-6			22.7	NS
12-7			98	343
12-8			49	260
12-9			22.7	NS
5-1	PRCV(O-IN)/TGEV(IM)	PRCV(O-IN)[4]	22.7	19
5-2			16	19
5-3			32	19
5-4			19	5.7
5-5			21	8.0
5-6			22.7	37
5-7			16	4.0
5-8			16	5.7
5-9			16	11.3
10-1			75	43
10-2			130	21
10-4			86	28
10-5			75	21
10-6			57	25
10-7			86	25
10-8			37	16
10-9			49	21
11-2			260	19
11-4			86	25
11-5			28	<2.0
11-7			33	25

1. DPC = Days Post PRCV Challenge

2. Piglets were challenged 2 ml orally with $10^{3.0}$ PID_{50} of TGEV at 14 days of age and 1 ml oral and 1 ml intranasally with $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days of age.

3. NS = No Sample

4. Pigets were challenged 1 ml oral and 1 ml intranasally with PRCV $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days of age.

Table 5 . Piglet serum neutralizing antibodies vs TGEV (Cont.)

Inoculations			Piglet serum virus titer	
Animal #	Gilts	Piglets	Day of PRCV Challenge	21 DPC[1]
6-1	Uninoculated	PRCV(O-IN)[4]	≤2.0	2.8
6-2			≤2.0	≤2.0
6-3			≤2.0	16
6-4			≤2.0	≤2.0
6-5			≤2.0	2.8
6-6			≤2.0	4.0
6-7			≤2.0	8.0
6-8			≤2.0	2.8
7-1			≤2.0	NS[3]
7-2			≤2.0	4.0
7-3			≤2.0	≤2.0
7-4			≤2.0	4.0
7-5			≤2.0	5.7
7-6			≤2.0	NS
7-7			≤2.0	≤2.0
7-9			≤2.0	≤2.0

1. DPC = Days Post PRCV Challenge
2. Piglets were challenged 2 ml orally with $10^{3.0}$ PID_{50} of TGEV at 14 days of age and 1 ml oral and 1 ml intranasally with $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days of age.
3. NS = No Sample
4. Piglets were challenged 1 ml oral and 1 ml intranasally with PRCV $10^{9.3}$ $TCID_{50}$/ml of PRCV at 28 days of age.

DISCUSSION

It has been thought by Coronavirus researchers that PRCV may be a suitable candidate for vaccination against TGEV infection (13). Publications have suggested PRCV may multiply in the gut and elicit TGEV neutralizing antibodies (5). It has been reported that pregnant sows (or gilts) exposed at the gut level to virulent TGEV will secrete TGEV neutralizing IgA antibodies in their milk (lactogenic immunity) (20). Fitzgerald et. al. have demonstrated that these animals can be boosted parenterally and in subsequent farrowings reproduce IgA levels seen in the initial infection. Therefore, it was decided to use this pregnant sow model to determine if PRCV recovered animals, boosted parenterally against TGEV, would respond in a similar fashion as those that had been primed with TGEV only.

One of the parameters used was to evaluate IgG and IgA imunoglobulin levels produced in colostrum, seven and fourteen day milks. The four groups used were non-

vaccinated controls, animals exposed to PRCV, animals vaccinated IM with TGEV, and animals which had recovered from PRCV infection and subsequently boosted IM with TGEV. Serum and milk neutralizing titers to TGEV were evaluated and no TGEV neutralizing titers were detected in the non-vaccinates. Neutralizing titers in colostra and sow sera had similar antibody levels and were not found to be significantly different between the PRCV and the PRCV/TGEV-IM groups (Table 2). Fourteen day milks were not significantly different between the three groups but were found to drop in titer 10-fold when compared to the colostra, suggesting an IgG type response. When these colostra and milks were evaluated for TGEV IgA, none was found (Table 3). These data demonstrate that PRCV does not significantly replicate in the proper tissues to allow for an IgA type secretion. This IgA secretion is important in considering a virus isolate as a vaccine candidate for the prevention of TGEV infections. All vaccinated groups had good TGEV IgG responses with the highest levels being observed in the TGEV-IM vaccinated group followed by the PRCV/TGEV-IM group and PRCV group, in descending order. Due to the high levels of IgG seen in the TGEV-IM group, relatively low levels of IgG were detected in seven-day milks whereas in all other groups, IgG levels were undetectable with the exception of one animal in the PRCV/TGEV-IM group. An increase in TGEV neutralizing antibodies was observed in some of the 28-day post-farrow sow sera. This was due to the fact that their litters were challenged with virulent TGEV and it became apparent that the sows themselves became exposed, thus further suggesting that the sows themselves were not protected from virulent TGEV by the viruses or routes used.

Litters from the non-vaccinated controls, TGEV-IM and PRCV groups were challenged at fourteen days of age with virulent TGEV. All animals developed a creamy to watery diarrhea that persisted for four to seven days. No protection was observed in any of these animals when compared to challenged piglets that were nursing non-vaccinated controls.

Using this sow model it was demonstrated that PRCV is not a good vaccine candidate for TGEV infections because an IgA type of antibody response was not produced, no passive protection was observed, and immunized sows themselves became infected. This phenomenon is similar to what happens when TGEV is administered parenterally (8). Without oral priming, antigen is not presented to the proper mucosal surfaces (Peyers patches) resulting in a systemic immunity that is not protective at the gut (mucosal) level.

Our data suggest that PRCV does not multiply at the gut level, therefore it is hypothesized that this virus is a strict respiratory pathogen. In order to more fully understand the mechanisms of protection via trafficking lymphocytes, it was decided to investigate whether gut level exposure to TGEV would confer protection to PRCV (respiratory) infections. Additionally, it was thought that if PRCV is solely a respiratory infection, protection should be seen in the piglets suckling PRCV or PRCV/TGEV-IM exposed gilts. The reason for this is that within the first 24-hours of birth, nursing piglets absorb, via the colostrum, IgG antibodies which will eventually enter the bloodstream and circulate systemically.

What was of interest was determining whether these type of antibodies would be protective when comparing piglets that had not suckled PRCV exposed gilts to piglets (who had also not suckled from PRCV exposed gilts) that had recovered from a virulent TGEV infection. This was demonstrated by inoculating these groups of piglets, along with piglets which had suckled from PRCV/TGEV exposed gilts, with PRCV. Piglets which suckled PRCV/TGEV positive dams and piglets (who had suckled from non-vaccinated dams) that had recovered from a virulent TGEV infection did not significantly shed virus during the post-challenge period. On the other hand, piglets which had suckled from non-vaccinated dams, significantly shed virus up to seven days post-challenge. This clearly demonstrates that piglets acquiring TGEV antibodies, actively or passively, will be protected from a PRCV infection. This protection also confirms our hypothesis that PRCV is a strict respiratory pathogen and that gut level exposure does confer protection against respiratory infections.

Since exposure to TGEV protects against PRCV infection, it was wondered if this subsequent exposure to PRCV would have any anti-TGEV booster effect. Piglets that had suckled from non-vaccinated gilts and had recovered from a TGEV infection prior to PRCV exposure, all had an average increase in anti-TGEV titers of eight-fold while piglets that had also suckled from non-vaccinated gilts had an average increase in anti-TGEV titers of three-fold. Piglets that had suckled from PRCV/TGEV gilts did not show any increase in anti-TGEV titers. This data show that TGEV vaccination is enhanced by exposure to PRCV. This is of particular interest in that PRCV continues to spread across the United States, Canada, and Europe.

In this study, and others previously done (21), it has been shown that prior PRCV infection does not protect against TGEV virulent virus challenge. This is due mainly to the fact that PRCV is a respiratory pathogen and does not replicate in gut mucosal surfaces.

This lack of gut mucosal priming results in the absence of long lasting, protective, neutralizing IgA antibodies which can be passed on to suckling piglets. What would also be missing would be the ability to boost this antibody response with a parenteral vaccination. This study further demonstrated that passive or active acquisition of TGEV antibodies, protect against PRCV infection. The class of antibodies that conferred this protection were of the circulating IgG type. Finally, it was shown in this study that TGEV antibodies are significantly boosted following exposure to PRCV. This fact may be of interest when more TGEV vaccines are developed, tested, and/or improved in the future.

CONCLUSIONS

Three conclusions can be made from this study. First, this study demonstrated that prior PRCV infection does not protect against TGEV virulent virus challenge. Second, it was shown that passive or active acquisition of TGEV antibodies protect against PRCV infection. Finally, it was observed that TGEV antibodies are significantly boosted following exposure to PRCV.

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