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Increased litter survival rates, reduced clinical illness and better lactogenic immunity against TGEV in gilts that were primed as neonates with porcine respiratory coronavirus (PRCV)☆

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Abstract

Establishing immunological memory in female piglets at a young age with PRCV was effective in inducing a secondary immune response to a limiting dose of virulent TGEV given orally 13–18 days prior to farrowing. Subsequently, because of passive antibody transfer, the offspring of these primed gilts were more efficient in surviving a lethal TGEV challenge. An average survival rate of 89% occurred in 6 litters of piglets from primed gilts that were boosted with 2.8×10^6 plaque forming units (PFU) of TGEV whereas 76% of the piglets survived in three litters that suckled primed gilts boosted with 3.0×10^5 PFU of TGEV. Non-primed gilts given identical pre-farrowing doses of TGEV had litter survival rates of 63 and 55%, respectively. Moreover, both groups of litters from primed gilts suffered less clinical illness (as measured by the extent of weight loss post-challenge) than control litters. Priming of the piglets as neonates and boosting the pregnant gilts produced an anamnestic systemic immune response and correspondingly higher milk titers in the primed gilts compared to control animals. Thus, priming piglets with PRCV was beneficial in providing resistance to TGEV and could be incorporated into a vaccine strategy that yields better protection against TGEV.

Keywords: Porcine respiratory coronavirus (PRCV); TGEV; Lactogenic immunity; Immunologic memory

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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1. Introduction

Transmissible gastroenteritis virus (TGEV) replicates in and destroys the mature absorptive epithelial cells of the small intestine causing villous atrophy and enteritis (Moon, 1978). TGE is often fatal in young suckling pigs because they have limited nutrient reserves and because villus enterocytes are slow to regenerate (Moon et al., 1975). In mature pigs, except perhaps for pregnant dams, TGEV infections are subclinical or occasionally soft feces may be observed. Other sites of TGEV replication include the respiratory tract and mammary gland but the virus is most easily isolated from intestinal contents and feces (Saif and Bohl, 1983; Saif and Wesley, 1999).

Pregnant sows and gilts are vaccinated for TGE, either prophylactically or during an acute disease outbreak, in order to passively immunize their suckling piglets by ingesting colostrum and postparturient milk containing protective TGEV antibodies (Hooper and Haelterman, 1966). The ingestion of colostral and milk antibodies that neutralize TGEV and thus protect the susceptible epithelial cells of the small intestine is termed lactogenic immunity (Haelterman, 1965). Frequent nursing by the piglets ensures a plentiful supply of passive antibody to neutralize the virus. Sows and gilts infected with virulent TGEV develop sufficient maternal antibodies (IgA and IgG) to protect nearly all of their offspring (Stone et al., 1977) while the efficacy of attenuated TGE vaccines is less efficient (Saif and Wesley, 1999). Moreover, the dose of virulent virus is important in establishing adequate lactogenic immunity (Wesley, 2000).

In contrast to TGEV, porcine respiratory coronavirus (PRCV) is isolated most readily from the upper and lower respiratory tract, and little if any virus replication occurs in the intestinal tract. PRC viruses are spontaneous deletion mutants of TGEV and these deletions have occurred with some frequency in Europe and in North America since the mid-1980s (Pensaert et al., 1986; Wesley et al., 1990; Britton et al., 1991; Vaughn et al., 1995; Kim et al., 2000). A notable change between TGEVs and PRCVs is a large deletion in the N-terminal portion of the PRCV spike or S glycoprotein leading to a truncated S protein which may account for the different tissue tropisms of these viruses (Krempl et al., 1997; Sanchez et al., 1999). Most of the antigenic sites on the PRCV S protein remain unchanged from those of the TGEV S protein so that an infection of pigs with PRCV induces similar neutralizing antibody titers for both PRCV and TGEV.

In terms of cross protection for pigs infected with PRCV, the results have been inconsistent. In one study using specific pathogen free (SPF) pigs, prior infection with PRCV caused no reduction in the amount or duration of shedding of TGEV (van Nieuwstadt et al., 1989). In contrast, a PRCV infection in weaned pigs of a similar age showed that PRCV-primed the humoral and local immune systems against TGEV and that a secondary antibody response was induced by the TGEV challenge (Cox et al., 1993). Since young piglets are most susceptible to TGEV, this heightened immunity, therefore, would be beneficial for the litters of primed female pigs. Thus, the purpose of this study was to measure increases in protective passive immunity against TGEV in gilts that were primed with PRCV when they were 3- and 4-day-old neonates (Wesley, 2002). These PRCV-primed gilts were boosted with a low oral dose of TGEV (doses that were insufficient to provide complete protection, Wesley, 2000) at 13–18 days prior to farrowing and their secondary antibody response and milk titers were measured. Also, litters of these primed and boosted gilts were challenged

with TGEV and the clinical severity of disease and the percentage of surviving piglets was determined.

2. Materials and methods

2.1. Animal history and experimental design

Piglets (principals) were born at the National Animal Disease Center (NADC) from serologically negative sows. Prior to infection the principals were serologically negative for TGEV/PRCV serum neutralizing (SN) antibodies and, at 3 and 4 days of age, they were infected intranasally with 6×10^6 plaque forming units (PFU) of the avirulent Ind/89 PRCV strain (Wesley, 2002). The PRCV-primed female piglets were maintained in an isolation room for 8 weeks and then commingled in a single outside pen with age-matched control females from another litter. The serological status of the pigs was followed while they matured and after they were bred to TGEV/PRCV serologically negative boars (Wesley, 2002). The experimental design using these principals was to stimulate lactogenic immunity by exposure to a low oral dose of TGEV before farrowing and then to determine whether the litters of the PRCV-primed gilts were better able to resist and to survive a challenge exposure to TGEV.

Approximately 2 weeks before farrowing, the nine primed and three control gilts plus five other control gilts (purchase from an outside commercial source) and one control first parity sow were brought into an isolation barn at the NADC to farrow. The dams were bled at 13–18 days before farrowing (Tables 1 and 2) and then given a low dose of virulent TGEV (Miller strain, pig-passaged only) as either a booster dose (in the case of the primed pigs) or as an initial dose in the case of the control animals. The TGE stock virus (2.8×10^6 PFU/ml) was diluted in modified Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS) and each gilt was inoculated with 12 ml (10 ml given orally and 1 ml given in each nostril). Two different doses of TGEV were used. Six of the primed gilts and

Table 1
Percent survival rate per litter and overall survival percentages (high dose)

Gilt number (primed)	Days before farrowing[a]	Litter survival rate	Gilt number (controls)	Days before farrowing[a]	Litter survival rate
1–1	18	100 (10/10) [b]	3–5	14	100 (6/6)
2–11[c]	18	100 (7/7)	042	15	78 (7/9)
2–3[c]	14	100 (12/12)	047	16	78 (7/9)
2–6	17	92 (11/12)	3–1	18	50 (3/6)
2–1[c]	14	86 (6/7)	045	16	50 (3/6)
1–3	16	50 (4/8)	3–2	17	30 (3/10)
Total survival		89 (50/56)[d]			63 (29/46)

[a] Gilts were given the high dose of TGEV at this time before farrowing.
[b] Survivors per total piglets in a litter are in parentheses.
[c] Primed gilts that were seronegative at the time the TGEV booster dose was given.
[d] $P < 0.05$.

Table 2
Survival percentage per litter and overall survival percentages (low dose)

Gilt number (Primed)	Days before farrowing[a]	Litter survival rate	Gilt number (controls)	Days before farrowing[a]	Litter survival rate
2–9	13	100 (3/3)[b]	048	17	90 (9/10)
2–7	15	78 (7/9)	046	17	75 (6/8)
2–2	18	68 (6/9)	102[c]	15	9 (1/11)
Total survival		76 (16/21)[d]			55 (16/29)

[a] Gilts were given the low dose of TGEV at this time before farrowing.
[b] Survivors per total piglets in a litter are in parentheses.
[c] First parity sow.
[d] $P < 0.45$.

six of the control gilts were inoculated with 2.8×10^6 PFU of TGEV (higher dose). Three of the primed gilts and three controls (two gilts and a first parity sow) were given 3.0×10^5 PFU of TGEV (lower dose). These are minimal doses to stimulate lactogenic immunity (Wesley, 2000). On the day of inoculation and daily for 7 days, body temperatures were determined and rectal swabs were taken from each animal. Also the dams were monitored twice daily for clinical signs including changes in stool consistency and inappetence. Serum samples from the dams and milk were collected on day 2 post-farrowing. Sera and milk were also collected on day 15 which was 12 days after the piglets were challenged with TGEV.

2.2. TGEV challenge of piglets

The nursing piglets were bled prior to TGEV challenge in order to determine maternal antibody levels that were transferred from the dams. At 3 days of age the piglets were challenged with approximately 500 pig-lethal-doses of the virulent Miller (p439) strain of TGEV (Wesley et al., 1988). Frozen aliquots of the challenge virus were thawed and diluted 10-fold in phosphate buffered saline. The virus was then sonicated briefly to disrupt viral aggregates. The sonicated challenge virus was diluted further in EMEM supplemented with 2% FBS to a final dilution of 10^{-3}. Each piglet was challenged with 5 ml of diluted TGEV directly into the stomach via a stomach tube. Starting at 3 days of age, piglets were weighed daily before challenge and for 12 consecutive days after challenge exposure. Piglets were observed three times daily for clinical signs and a post-challenge exposure interval of 12 days was used to determine pig survival rates.

2.3. Virus neutralization assays

Milk whey and serum samples from the dams were heat inactivated and the TGEV/PRCV neutralizing antibody titer was determined by a plaque-reduction test using swine testicular (ST) cells (Woods et al., 1988). An attenuated, plaque-purified Miller strain of TGEV, passed twice on ST cells, was used for this assay. Approximately 100 PFU of attenuated TGEV was incubated for 1 h at 37 °C with 2-fold dilutions of test sample before inoculating and overlaying ST cells. The virus neutralizing (VN) antibody titer was expressed as the reciprocal of the highest dilution that produced 50% reduction in plaques.

Sera from the piglets also were heat inactivated and the TGEV/PRCV neutralizing antibody titer was determined by a VN test using a 96-well flat-bottomed microtiter plate with confluent ST cells (Paton et al., 1996). Two-fold serum dilutions were tested using four wells per dilution. After incubating the plates for 7 days at 37 °C in a humidified 3% CO_2 atmosphere, the cells were fixed with methanol and stained with crystal violet. Serum neutralizing titers were determined by the Spearman–Karber method at the dilution of serum that neutralized attenuated TGEV in 50% of the wells.

2.4. TGEV recovery from rectal swabs

Rectal swab samples were collected from the dams prior to giving the pre-farrowing dose of TGEV (either a booster or an initial dose) and for the 7 days following the TGEV exposure. Rectal swabs were placed in 1 ml of cold EMEM supplemented with FBS (20%), penicillin (25 U/ml), streptomycin (25 ug/ml), neomycin sulfate (25 ug/ml), bacitracin (0.25 U/ml), gentamicin sulfate (50 ug/ml) and stored frozen at −70 °C. Swab samples were thawed, squeezed with sterile forceps, and centrifuged at 4 °C in a microcentrifuge for 6 min to remove debris and to reduce bacterial load. Two hundred microliters of each sample supernatant was applied to confluent ST cells on a 6-well plate. The inoculum was removed after 1 h and 4 ml per well of EMEM containing FBS (2%), antibiotics at concentrations listed above, porcine antisera against enterovirus and rotavirus (0.1%), and fungizone (2.5 μg/ml) was added. The plates were incubated at 37 °C in a humidified CO_2 incubator and then observed by light microscopy for cytopathic effect (CPE) after 5 days. Each swab sample was passed a total of three times on ST cells to ensure detection of TGEV. As positive controls for each test, 5 and 50 PFU of virulent TGEV (in 200 μl) was used to confirm the sensitivity of the ST cells for the Miller gut virus. The one sample causing CPE was confirmed as TGEV using an indirect fluorescent antibody test with a TGEV-specific monoclonal antibody.

2.5. Statistical analysis

The survival rate for each group of piglets was analyzed by computing a Chi-square statistic. Because litter sizes are not fixed, the method of Rao and Scott (1992) was used to account for litter size variation, and effectively, to adjust the underlying sample sizes. Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Induction of lactogenic immunity

Thirteen to 18 days before farrowing (Tables 1 and 2), primed gilts and control gilts were given virulent TGEV to stimulate lactogenic immunity. In a previous experiment it was determined that low oral doses of TGEV were adequate to stimulate lactogenic immunity in control gilts but provided limited protection for their litters (Wesley, 2000). At the time that the pre-farrowing dose was given, all of the control animals were negative for TGEV/PRCV

neutralizing antibody, 3 of the primed gilts were seronegative and the other 6 primed gilts had low SN titers of 12 or less (Wesley, 2002).

3.2. Clinical response

Following exposure to TGEV many of the gilts went off-feed for a few days and of these animals some had transient soft feces or a transient diarrhea. With the higher dose (2.8×10^6 PFU dose of TGEV), all of the primed gilts and three of the six control gilts were off feed while three of the primed gilts and two of the control gilts had soft stools or diarrhea. Similarly at the lower dose (3.0×10^5 PFU of TGEV) the primed gilts appeared to be more sensitive to the TGEV than were the control animals. Two of the three primed gilts went off feed whereas none of the gilts nor the first parity sow given the lower dose were off feed. No animals given the lower dose of TGEV show signs of diarrhea or soft feces. Furthermore, body temperatures and rectal swabs were taken daily for primed and control animals during the 7-day period following the exposure to TGEV. None of the animals were febrile and TGEV was recovered from only one fecal sample on a single day (day 3) post-exposure. Virus recovery was from 1 primed gilt (#2–2) given the lower dose of TGEV that showed no signs of diarrhea. No TGEV was recovered from rectal swabs of control gilts.

3.3. Serological response

Sera and milk were collected from primed and control gilts on day 2 post-farrowing. This represented a range of 15–20 days after exposure to TGEV. The geometric mean SN antibody titers and the geometric mean VN antibody titers of milk for each group of gilts are summarized in Fig. 1. These titers demonstrate that the primed gilts had an anamnestic immune response to the booster dose of TGEV compared to the control gilts given the same pre-farrowing dose as their initial exposure. The geometric mean SN titers were >10-fold greater in primed versus control animals on average for groups of gilts given either the higher or lower dose of TGEV. The milk VN titers showed the same trend but the difference between primed and control gilts was less. For the high dose there was, on average, a 5.4-fold increase and for the low dose an 8.0-fold increase in milk VN titer in the primed gilts compared to the geometric mean values for the control gilts. There was also a dose effect based on the exposure to TGEV. Both the primed and control groups of gilts given the higher dose of TGEV induced higher levels of neutralizing antibody on average than their corresponding groups given the lower dose.

Further, the post-farrowing (day 2) SN and milk VN titers were compared with their post-challenge SN and milk VN titers (day 11 or 12 post-challenge) to determine if the dams were re-infected by contact exposure with their TGEV challenged litters. For all the primed animals (given either the high or low dose), their SN and milk VN titers were lower on day 12 post-challenge indicating that they resisted re-infection (data not shown). The results with the control gilts were more variable. For the six control gilts given the higher dose, three of the SN titers decreased post-challenge, two increased and one stayed about the same. For these high dose control gilts, four of the milk VN titers decreased and two stayed about unchanged. In terms of SN titers for the three low dose controls, two increased

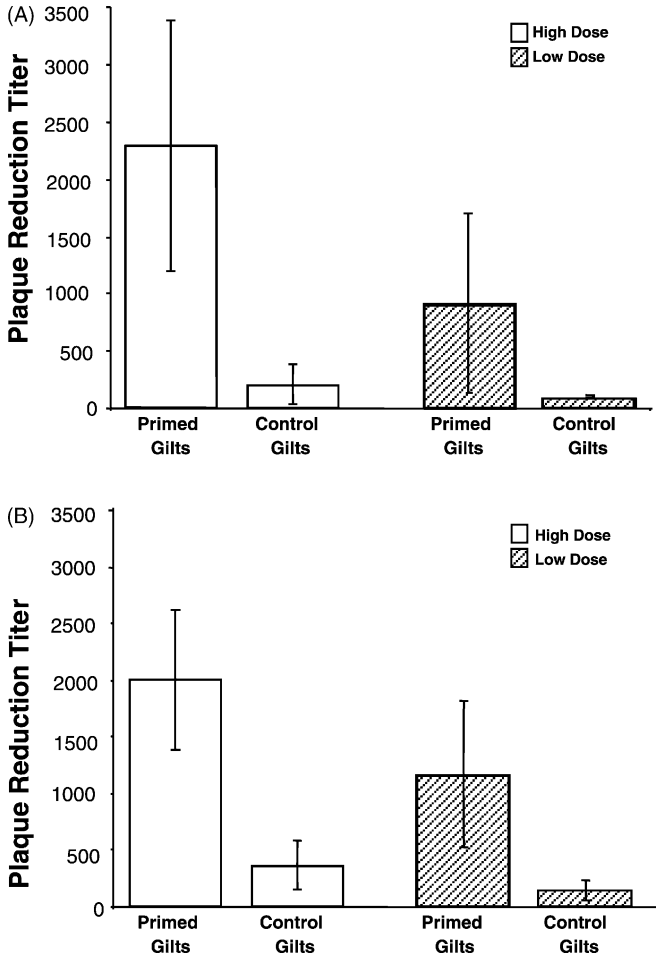

Fig. 1. Serum and milk virus neutralization titers. (A) Geometric mean serum neutralizing antibody titers and standard deviations are shown (high dose, primed gilts = 2299 ± 1093; high dose, control gilts = 206 ± 173; low dose, primed gilts = 916 ± 786; low dose, control gilts 83 ± 10). (B) Geometric mean virus neutralizing antibody titers and standard deviations in milk at day 2 post-farrowing were: high dose, primed gilts = 2004 ± 617; high dose, control gilts = 374 ± 221; low dose, primed gilts = 1176 ± 645 and low dose, control gilts = 146 ± 93.

post-challenge and one was unchanged whereas for the milk VN titers one decreased, one increased and one remained unchanged.

3.4. Maternal antibody transfer

Maternal antibodies are passively transferred and absorbed by the piglets for about 24–36 h after farrowing (Roth, 1999). SN titers on day 3 post-farrowing were determined for each piglet just prior to challenge with TGEV and the geometric mean titer for each

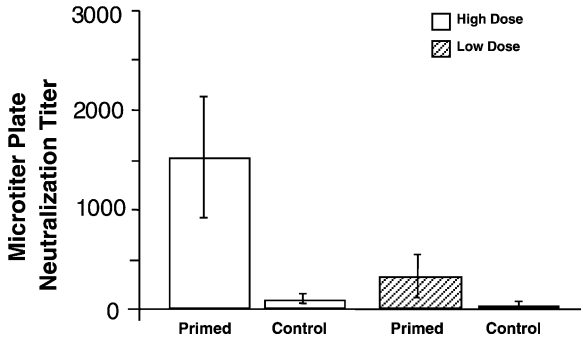

Fig. 2. Pre-challenge neutralization titers. Geometric mean serum neutralization titers for the litters of PRCV-primed and control gilts.

litter was determined. Fig. 2 shows the average and standard derivation of the SN titers for groups of litters from high and low dose primed and control gilts. The results are similar to the gilt SN titers in Fig. 1. Litters from high dose, primed gilts had the highest geometric mean titers (1511 ± 607), followed by litters from low dose, primed gilts (320 ± 178), then litters of control gilts given the higher dose (76 ± 59), and finally, litters of control gilts given the low dose had geometric mean SN titers of 10 ± 4.

3.5. Clinical response of the litters to TGEV challenge

Litters from both primed and control gilts showed varying degrees of enteric illness following challenge with TGEV. Individual piglets were weighed for 12 days post-challenge to monitor the severity of clinical infection and the recovery from infection. Average weights for each litter were calculated. The average daily weights showed that the litters from primed gilts given the high booster dose outperformed the litters from primed gilts given the lower dose and both groups of litters from primed gilts outperformed their corresponding control litters (Fig. 3). Litters from high dose, primed gilts on average gained weight for 2 days after challenge then lost weight from days 3 to 6 and then began to recover after day 6. High and low dose control litters and litters from primed gilts given the low booster dose showed much more severe weight losses on average than the litters of high dose, primed gilts.

3.6. Litter survival rates

Tables 1 and 2 show the survival rates for the litters of each experimental group. Litters from primed gilts boosted with the high dose had the greatest percent survivors (89%). Survival rates for this group were significantly improved ($P < 0.05$) compared to the high dose control group even after accounting for litter size variation (Rao and Scott, 1992). The low dose, primed gilts has the next best survival rate (76%) followed by the control groups exposed to either a high pre-farrowing dose (63% survival) or a low pre-farrowing dose (55%). The latter group consisted of litters of only three dams; two had good survival values whereas the one litter of the first parity sow markedly reduced the average survival rate for this group.

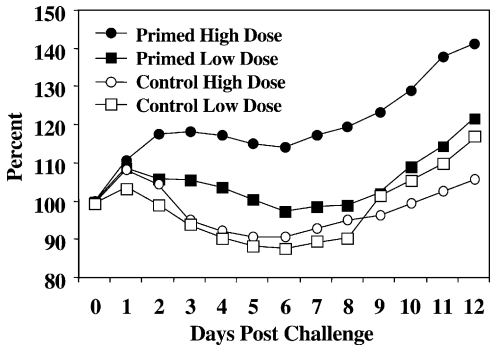

Fig. 3. Post-challenge average daily weights for each group of piglets. Geometric mean weights of piglets from PRCV-primed gilts given the high booster dose (●) or the low booster dose (■) of TGEV compared with the geometric mean weights of piglets of control gilts given the high pre-farrowing dose of TGEV (○) or the low pre-farrowing dose of TGEV (□).

4. Discussion

The serological data indicated clearly that "priming" 3- and 4-day-old female piglets by exposure to PRCV was effective in establishing protective immunological memory against TGEV. Immunological memory, that lasted at least through the first parturition of these pigs (∼1 year), was shown by an anamnestic SN antibody response and an enhanced lactogenic response in both groups of primed gilts. The antibodies measured in milk were total VN antibodies and no specific measurement of sIgA was made. All primed gilts given either booster dose had a greater than 10-fold increase in SN titers in comparison to their corresponding control groups. Likewise, there was an anamnestic VN response in the milk titers of primed gilts but these increases were less than for the SN titers. On average, there was a 5- to 8-fold increase in milk VN titers of primed gilts compared to the primary VN response in the milk of control dams. Moreover, primed gilts had better immunity since they were efficient in resisting re-infection with TGEV due to contact exposure to their TGEV challenged litter. No primed gilts show seroconversion at 12 days post-challenge whereas the control dams had variable results, some having declining titers while others had stable or increasing titers due to re-infection. Thus, by infecting young pigs with PRCV, immunological memory is induced that is long lasting and can be beneficial in protecting pigs and piglets from TGEV.

One possible negative aspect of the immunological memory was that primed gilts appeared to have more pronounced clinical signs to the oral dose of TGEV than did the naïve control dams. This observation simply could be in error because of the limited number of animals in the study. Indeed, in a previous experiment, non-primed gilts experienced inappetence but no diarrhea when given the same TGEV inoculum in a similar dose range (Wesley, 2000). Moreover, pigs having recovered from TGEV are resistant to subsequent re-infection with the virus, and thus, lack clinical signs (Cox et al., 1993; VanCott et al., 1994; Saif and Wesley, 1999). However, the duration of this resistance has not been reported for the approximate 1-year time interval used in this study. Perhaps one possibility with

PRCV-primed gilts is that the booster dose of TGEV may have an additional inflammatory component in the intestinal tract resulting in more apparent clinical signs. In this regard a respiratory infection with PRCV has been shown to stimulate antibody secreting cells that not only localize to the respiratory tract but also lodge in the gut associated lymphoid tissue (VanCott et al., 1994).

In a previous study, PRCV was used to inoculate 2-, 4- and 6-day-old piglets to induce active immunity (Wesley and Woods, 1996). No clinical signs of respiratory disease were observed in these neonatal animals other than a transient reduced weight gain. In these piglets partial protective immunity was established in 6 days and SN antibodies also were detected by day 6 post-exposure to PRCV. In the current study neonatal piglets were infected similarly with PRCV and SN titers of the primed pigs decayed over the course of the year following infection. These pigs had immunological memory since they developed a secondary immune response after booster exposure to TGEV and they transferred passive protective antibodies to their offspring. Additionally, Lanza et al. (1995) and Sestak et al. (1996) have shown that PRCV can induce immunological memory in older pigs. Sows in these studies were immunized with PRCV prior to their first parturition and again before their second parturition. These sows retained immunologic memory because there was a secondary humoral and lactogenic immune response, particularly increases in sIgA, that yielded better protection for their TGEV challenged offspring.

Immunologic priming of the common immune system with PRCV was reported in 5-week-old, weaned SPF pigs and in 4- to 6-week-old, weaned pigs conventionally raised (van Nieuwstadt et al., 1989; Cox et al., 1993). In both studies, priming was followed 4 weeks later by a virulent challenge with TGEV and an anamnestic humoral immune response occurred. In the latter study, an intestinal sIgA response in PRCV immune pigs suggested that local secondary immunity occurred in the intestinal mucosa which either completely blocked TGEV shedding in a few pigs or reduced the duration of shedding in other pigs.

Litter survival rates were quite variable which is not uncommon for TGEV challenge experiments with suckling piglets. For the litters of PRCV-primed gilts, the percent survival ranged from 50 to 100% at the higher dose and from 68 to 100% for only three litters at the lower dose. The survival rates in corresponding control litters ranged from 30 to 100% at the higher dose and from 9 to 90% at the lower dose. Chi-square probability distributions were used to evaluate the overall survival percentages. Even when litter size variations were taken into account (Rao and Scott, 1992), the overall percent survival of litters from PRCV-primed gilts was significant ($P < 0.05$) when compared to the survival rates of corresponding control litters. In contrast, at the lower booster dose, 3×10^5 PFU, survival rates for litters of primed versus control gilts were not significant ($P < 0.45$).

In conclusion, PRCV primes the immune response to TGEV and, in the current study, immunological memory occurred in gilts that were primed with PRCV even as young piglets. Thus, in the primed dams, the threshold of TGEV needed to induce protective immunity for their offspring is lower. Most likely, even a commercial, modified-live TGEV vaccine given orally to PRCV-primed gilts would provide superior protection for their offspring via lactogenic immunity.

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