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Immunization of pregnant gilts with PRCV induces lactogenic immunity for protection of nursing piglets from challenge with TGEV

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ABSTRACT

The level of passive protection against transmissible gastroenteritis virus (TGEV) was evaluated by experimentally infecting 12 pregnant gilts with different doses of porcine respiratory coronavirus (PRCV) and challenging their litters at 4 days of age. An overall survival rate of 70% was found for piglets nursing the 12 PRCV-infected gilts, compared to a 16% survival rate for piglets of nine uninfected control gilts. Six of the PRCV-infected gilts had adequate levels of immunity to resist infection with TGEV following the challenge of their litters. These six completely immuned gilts also solidly protected their litters from TGEV as shown by a 96% piglet survival rate through weaning at 3 weeks of age. The results suggest that respiratory infection with PRCV induces a substantial degree of protective lactogenic immunity against TGEV.

Key words: Transmissible gastroenteritis virus; Porcine respiratory coronavirus; Pig; Immunity; Vaccination

INTRODUCTION

Porcine respiratory coronavirus (PRCV), a deletion mutant of transmissible gastroenteritis virus (TGEV), was first isolated in 1984 (Pensaert et al., 1986). Since then the virus has spread by contact and by aerosol throughout much of Europe (Sánchez et al., 1992; Cox et al., 1993). TGEV challenge studies for piglets nursing sows immunized with PRCV suggest widely varying results (Bernard et al., 1989; Paton and Brown, 1990; De Diego et al., 1992). However, field observations indicate that the incidence of TGEV has declined in European countries concomitantly with the spread of PRCV.

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In 1989, another PRCV was isolated from three swine herds located at geographically distant sites in the midwest and the east coast of the United States (Wesley et al., 1990). When compared with a European PRCV strain, the U.S. PRCV had a similar but non-identical deletion in the spike protein gene (Rasschaert et al., 1990; Wesley et al., 1991). Thus, the U.S. PRCV is a new TGEV variant and did not spread to the United States from Europe.

Although genetically different, phenotypically, the U.S. and European strains of PRCV are similar in that both have a tropism for respiratory tract tissue and both lack the ability to infect and destroy swine enterocytes and to cause enteric disease. However, unlike the rapid dissemination of PRCV in Europe, PRCV in the United States has not spread widely as shown by a 1990 U.S. national swine survey (USDA, APHIS, 1991). That survey indicated that 36% of U.S. swine herds were serologically positive for TGEV antibody, a lower incidence than that found in a survey of midwestern swine in the early 1980s (Egan et al., 1982).

The purpose of this paper was to determine if the U.S. and European PRCV strains induce comparable levels of passive protective immunity to TGEV. In this study, pregnant gilts were vaccinated with the U.S. strain of PRCV. The results are discussed and compared to the protective immunity induced by the European PRCV strains.

MATERIALS AND METHODS

Virus

The PRCV used in these studies was isolated originally from nasal swabs taken from weaned pigs. This isolate has been referred to as PRCV-Ind/89 (Wesley et al., 1990) or ISU-1 (Hill et al., 1990). The PRCV was plaque-picked once on swine testicular (ST) cells. PRCV inocula were prepared in ST cells by two passages at a low multiplicity of infection. Usually, the inocula were concentrated by centrifugation at 28000 rpm, 3 h, 4°C in a Beckman SW28 rotor. The pelleted virus was resuspended in 5 ml F-15 medium (Gibco), titrated, and stored at −70°C. For some experiments, cell-free virus supernatant that had not been concentrated was used to inoculate gilts.

Animals and experimental design

Twenty-one pregnant gilts, serologically negative for TGEV antibody, were used in this study. Each gilt was housed separately in an individual isolation room. Nine gilts served as uninfected controls and 12 gilts were vaccinated with PRCV at the times and doses indicated in Table 1. PRCV was given to the pregnant gilts at 8, 4, and 2 weeks before farrowing. The initial two doses were given orally/intranasally (O/IN), while the third dose was divided — a portion given O/IN and a portion emulsified with Freund's incomplete adjuvant (Difco) and given intramuscularly (IM). Group I gilts received the

TABLE 1

Pre-farrowing vaccination protocol with PRCV

Time[1] (weeks)	Route[2]	Dose[3]		
		I	II	III[4]
8	O/IN	2×10^9	2×10^8	10^8
4	O/IN	2×10^9	2×10^8	10^8
2	O/IN	1×10^9	8×10^7	8×10^7
	IM	4×10^8	5×10^7	2×10^7

[1]Number of weeks before farrowing.
[2]O = oral; IN = intranasal; IM = intramuscular.
[3]Dose given in plaque forming units (PFU) to each group of gilts. Group I ($n=6$ gilts); Groups II and III ($n=3$ gilts)
[4]Litters of group III gilts were given 5×10^6 PFU of PRCV at 1 day of age.

higher doses of PRCV (Table 1). Gilts in Groups II and III received approximately 10-fold less virus at each exposure and the piglets of Group III gilts also received 5×10^6 plaque forming units (PFU) of PRCV intranasally (IN) at 1 day of age. No respiratory problems were observed in the PRCV-inoculated Group III piglets.

TGEV challenge

Four-day-old piglets nursing either vaccinated or control gilts were challenged with approximately 500 pig-lethal-doses of the virulent Miller (p439) strain of TGEV (Wesley et al., 1988). The challenge virus, prepared as an intestinal homogenate, was briefly sonicated to dissociate aggregates and diluted in cold F-15 medium containing 2% fetal bovine serum. Each piglet was challenged with 5 ml of diluted TGEV directly into the stomach via a tube.

Clinical evaluation

Following challenge exposure, each piglet was weighed daily and the body temperature of each gilt was measured daily. Clinical signs for both gilts and piglets were recorded twice daily. Surviving piglets, for survival rate determinations, were those animals alive on day 19 post challenge (PC).

Virus neutralization

Serum samples from blood collected during the vaccination protocol, colostrum collected within 12 h post-farrowing, and milk collected on post-challenge day 10 were tested for TGEV/PRCV neutralizing antibody. The virus-neutralizing (VN) antibody titer is reported as the reciprocal of the serum dilution causing a TGEV plaque reduction of 50% on ST cells (Woods et al., 1988).

Statistical analysis

Survival rates, VN titers, and daily weight gains were each compared by the analysis of variance (the F-test). Differences with $P<0.05$ were considered to be significant.

RESULTS

Serological response of gilts to PRCV

The serum neutralization (SN) titers of 12 gilts following the vaccination protocol with PRCV are shown in Table 2. The SN titers at 4 weeks following the primary exposure to PRCV ranged from 241–3090 with a geometric mean, $\bar{x}=1142\pm231$. There was no correlation between the initial oronasal virus dose and the magnitude of the SN response. A second oronasal exposure to PRCV did not stimulate SN antibody levels. However, the third vaccination with PRCV, in which the dose was split O/IN and IM with Freund's incomplete adjuvant, did increase the SN titer of each gilt, $\bar{x}=3050\pm420$ ($P=0.0001$).

Litters: Survival rate, weight gain, clinical response

Litters from the PRCV-vaccinated gilts ($n=12$) and the unvaccinated control gilts ($n=9$) were challenged with virulent TGEV at 4 days of age. The survival rate of litters from gilts given the higher PRCV dose (Group I) or

TABLE 2

Serological response of naive gilts to PRCV

Group No.	Gilt No.	Pre-PRCV exposure	4 wks post-infection	2 wks post-2nd-infection	2 wks post-3rd-infection
I. (2×10^9 PFU)[1]	106	Neg	1820	2037	2455
	107	Neg	632	652	1432
	108	Neg	1361	718	3589
	162	Neg	518	1047	3467
	118	Neg	1213	1202	1479
	161	Neg	1844	1778	4898
II. (2×10^8 PFU)	157	Neg	3090	3388	4571
	144	Neg	550	1072	1380
	163	Neg	562	912	2951
III. (1×10^8 PFU)	6	Neg	977	596	5962
	10	Neg	900	664	2806
	P-7	Neg	241	205	2669
Mean ($\bar{x}\pm$S.E.)		–	1142 ± 231	1189 ± 248	3053 ± 420

[1]Initial PRCV dose.

approximately 10-fold less PRCV (Groups II and III) were similar (Table 3). Infecting baby piglets at 1 day of age with PRCV (Group III) and challenging with TGEV at 4 days of age did not improve the litter survival rate. The combined survival rate of litters from PRCV-vaccinated gilts was 70% (72/103 piglets). In contrast, the survival rate of litters from seronegative control gilts was 16% (11/68 piglets).

By 1 day post-challenge (PC), litters of both the PRCV-vaccinated gilts and the control gilts ceased gaining weight (Table 4). On average, litters of control gilts lost weight more rapidly than those of PRCV-vaccinated gilts; however, only differences in the day 1 PC weights were significant at the 95% confidence level ($P=0.04$).

All litters exposed to TGEV developed clinical signs of vomiting and diarrhea within 1–2 days PC. Following these early signs, piglets of PRCV-vaccinated gilts often had a "downy", bloated appearance during days 2–5 PC, and most of these piglets survived. In contrast, most of the piglets of control gilts were dehydrated, gaunt, weak, and died between days 3–7 PC.

TABLE 3

Number of piglets from litters of PRCV immunized or control gilts surviving challenge with TGEV at 4 days of age

Group No.	PRCV-vaccinated gilts			Control gilts		
	Gilt No.	Survivors/ total	Survival rate	Gilt No.	Survivors/ total	Survival rate
I (2×10^9 PFU)[1]	106	9/9		153	3/11	
	107	3/3		142	1/9	
	108	9/9		143	2/9	
				155	1/5	
	162	8/8		9	0/10	
	118	4/12		30	0/6	
	161	0/8	67%	31	0/3	
				32	0/6	
				789	4/9	16% (11/68)
II (2×10^8 PFU)	157	9/10				
	144	6/10				
	163	5/8	71%			
III[2]	6	7/8				
	10	7/9				
	P-7	5/9	73%			
	Total		70% (72/103)			

[1]Initial PRCV dose.
[2]One-day-old piglets of group III gilts were exposed to PRCV.

TABLE 4

Litter weights as a percentage of challenge day weight

	PRCV-vaccinated gilts					Control gilts		
Gilt No.	0	1[1]	2	3	Gilt No.	1	2	3
162	100	107	107	109	142	94	89	84
118	100	97	94	87	143	100	95	91
161	100	101	93	88	155	97	88	87[2]
157	100	109	112	111	9	100	91[2]	86[2]
144	100	104	97	92	30	94	90	84[2]
163		99	98	93	31	104	99	102[2]
6		101	93	89	32	99	90[2]	87[2]
10		106	101	96				
P-7		98	91	87	789	98	98	99[2]
Mean ($\bar{x}$) =		102	98	95	$\bar{x}$=	98	93	90

[1]Days post-challenge
[2]Values normalized because one or two piglets died on the day indicated

Gilts: Susceptibility or resistance to TGEV exposure

All nine of the control gilts were secondarily infected with TGEV following challenge of their litters because each gilt seroconverted by 10 days PC (data not shown). No clinical signs of TGEV infection, eg. soft feces or diarrhea, were observed in control gilts; however, seven of nine gilts had an elevated temperature ($\geqslant 40°C$) for one day between 2–5 days PC.

Half (6/12) of the PRCV-vaccinated gilts did not become infected by exposure to TGEV as shown by the lack of an anamnestic serological response (Table 5). These six completely immuned gilts (#106,107,108,162,157,6) also showed solid protection of their litters, 96% survival (45/47 piglets). Two PRCV-vaccinated gilts (#10,P7) had soft stools PC, and gilt #163 had a pronounced diarrhea lasting for 1 day. Three PRCV-vaccinated gilts (#10,163,118) had an elevated temperature for 1 day either on the second or third day PC. Four of the PRCV-vaccinated gilts showed a strong anamnestic response to TGEV (Table 5) with a four-fold or greater increase in SN titer. Two gilts had a less pronounced serological response to TGEV exposure. Low milk recovery at day 10 PC suggested that gilt #161 may have become agalactic during the PC period, and thus, lost piglets to starvation rather than to TGEV infection.

Colostral VN titers did not correlate with litter survival (Table 6). Also, the change in the VN titer between colostrum and 10-day milk sample was not as indicative of protection from challenge as were the serological changes described above. Of the six protected gilts, five had lower VN titers in the 10-day milk samples than in the colostral samples, and the VN titer of gilt #6

TABLE 5

Serological response of PRCV vaccinated gilts to TGEV challenge of their litter

	Gilt No.	Prechallenge	10 Days post-exposure
I	106	2455	1875
	107	1432	1914
	108	3589	2000
	162	3467	4786
	118	1479	63 096
	161	4898	16 596
II	157	4571	3715
	144	1380	3470
	163	2951	47 863
III	6	5962	5561
	10	2806	21 365
	P-7	2669	24 832

TABLE 6

Neutralizing antibody titer in colostrum and milk of gilts immunized with PRCV

	Gilt No.	Colostrum	10 Day Milk
I	106	5546	716
	107	14 388	355
	108	13 152	1091
	162	16 982	3090
	118	11 749	19 055
	161	4571	14 454
II	157	24 547	2188
	144	4365	2951
	163	8913	4266
III	6	12 791	12 733
	10	30 126	15 382
	P-7	8608	21 618

was unchanged. However, the VN titers also declined in the milk samples of three of the PRCV-vaccinated gilts that became infected secondarily with TGEV.

DISCUSSION

Our results indicate that experimental vaccination of seronegative, naive gilts with PRCV induces lactogenic immunity against TGE. The use of first-litter gilts in these experiments is the most stringent test for colostral immunity because the possibility of a previously undetected exposure to TGEV is reduced. Gilts were experimentally infected with different doses of PRCV to determine the level of cross-protection, and their litters were uniformly challenged with TGEV at 4 days of age. To stimulate a maximum level of passive immunity, the gilts were immunized three times at 8, 4, and 2 weeks prior to farrowing. The last vaccination was divided between the O/IN and IM routes, the latter emulsified with adjuvant to further stimulate immunity. Passively acquired cross-protection to TGEV by vaccination with PRCV was significant ($P=0.0001$), but varied among the litters. The overall survival rate was 70% of 103 piglets farrowed by 12 PRCV-vaccinated gilts, compared to a 16% survival rate of 68 TGEV challenged piglets from nine control gilts. Within a litter, the survivors ranged from 100% (4 litters) to 0% (1 litter), although piglets from the litter with a 0% survival rate could possibly have died from starvation due to agalactia caused by secondary infection of the gilt with TGEV.

Of the 12 PRCV-vaccinated gilts, six were completely immuned against secondary TGEV infection. Litters from these six immuned gilts had a survival rate of 96% (45/47 piglets). Resistance to infection and passive protection were probably due to the fact that these gilts produced and transmitted to their suckling piglets the highest levels of protective antibody. The other six PRCV-vaccinated gilts were infected with TGEV, as shown by increased SN titers following challenge. Two of the vaccinated gilts had soft stools following challenge and a third had a pronounced but transient diarrhea. Thus, gilts vaccinated with the U.S. strain of PRCV were not completely protected against TGEV. Similar observations were made by Hooyberghs et al. (1988) in Belgium where TGEV outbreaks occurred in swine herds that were naturally exposed to the European isolate of PRCV.

The level of VN antibody in serum and colostrum that was induced by our PRCV vaccination protocol did not correlate with piglet survivability. The lack of correlation between these parameters was reported previously for PRCV (Bernard et al., 1989) and has been recognized as a consistent feature of TGEV vaccination and challenge experiments (Saif and Wesley, 1992). This suggests that the immunodominant neutralizing epitopes for PRCV and TGEV are probably not the major contributors to passive protection. Our results also show that the initial PRCV dose, in the range of 10^8–2×10^9 PFU, did not correlate with the magnitude of the primary immune response. Thus, the degree to which PRCV replicates in the respiratory tract and induces immunity is probably more dependent on host factors and the virus strain rather

than on the initial virus dose. The second booster dose did not increase the humoral immune response, but the third dose did ($P=0.0001$), presumably due to the emulsification with adjuvant and the IM route. This increase in immunity from the third dose by the IM route may explain why antibody against the U.S. PRCV protected piglets better than previously reported results with sows exposed to European PRCV isolates.

Studies to measure the level of passive protection induced in sows with European PRCV isolates have yielded variable results. In studies by Paton and Brown (1990) and by De Diego et al. (1992), seronegative sows were experimentally infected with either a low or high dose of PRCV, respectively. In both instances, following two oronasal exposures to PRCV, the serum and colostrum VN titers were approximately 10-fold less than we report here following infection with the U.S. PRCV. Paton and Brown (1990), using a challenge TGEV of low virulence, concluded that PRCV-vaccinated sows transmitted no passive protection to their nursing piglets, whereas De Diego et al. (1992) protected 47% (7/15) of the piglets from two PRCV-vaccinated sows. Bernard et al. (1989) also tested passive protection against TGEV in sows naturally infected with PRCV. Serum titers in these sows were also low because of the >1 year time interval since exposure to PRCV. With these naturally infected sows and using a virulent challenge virus, Bernard et al. (1989) protected 56% of the piglets. The survival rate among these litters was highly variable; two sows protected all piglets in their litters and two sows did not protect any of their piglets. Our results support the observations of Bernard et al. (1989) and De Diego et al. (1992) and show that there is a link between respiratory infection with PRCV and secreted protective antibody in the mammary glands of post-parturient gilts. Since the serum and colostral VN titers are lower following vaccination with European isolates, it is possible that the European PRCVs could have lower infectivity for sows and gilts than the U.S. PRCV isolate.

In the United States, TGE is still a significant problem. In Europe, the spread of PRCV has apparently eliminated TGEV as a significant disease, probably by reducing the number of susceptible pigs. Why PRCV has not spread similarly in the United States is not known. The results of this study and the situation in Europe indicate that inoculating swine with PRCV would be beneficial in the control of TGE. Efforts to vaccinate with PRCV alone or to combine PRCV with attenuated enteric TGEVs should help in eliminating TGE as a disease problem.

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