Intestinal protection against challenge with transmissible gastroenteritis virus of pigs immune after infection with the porcine respiratory coronavirus

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An infection of pigs with the porcine respiratory coronavirus (PRCV) induces antibodies which neutralize the enteropathogenic transmissible gastroenteritis virus (TGEV) and PRCV to the same titre. In the present study, 10-week-old seronegative pigs (n = 8), *pigs immune following TGEV inoculation* (n = 4) *or pigs immune following aerosol* (n = 8) *or intragastric inoculation* (n = 4) *with PRCV were challenged with TGEV. Whereas TGEV-immune pigs were completely protected against challenge, all PRCV-immune pigs showed serological evidence of TGEV replication. Nevertheless, the aerosol or intragastric inoculation with PRCV primed the humoral immune system against TGEV and the TGEV challenge induced a secondary antibody response in most PRCV-immune pigs. Furthermore, all PRCV-immune pigs showed a decrease in the duration of the excretion of infectious TGEV (0–4 days) in comparison with the duration of the virus excretion by seronegative pigs (5–6 days).*

Keywords: Transmissible gastroenteritis virus; porcine respiratory coronavirus; challenge; protection; immune response

INTRODUCTION

Since its appearance in 1984, the porcine respiratory coronavirus (PRCV) has become widespread in the European swine population. This virus is antigenically closely related to the enteropathogenic coronavirus, transmissible gastroenteritis virus (TGEV)[1]. An infection of pigs with PRCV induces antibodies which neutralize TGEV and PRCV to the same titre[2]. Infections with PRCV and TGEV cannot be distinguished by the classical seroneutralization test. Using monoclonal antibodies against different TGEV-protein antigenic sites, it was shown that TGEV and PRCV have similar epitopes in the M and N proteins and in the neutralization-mediating antigenic sites of the S protein. However, an S antigenic site which stimulates non-neutralizing antibodies was observed to differ between both viruses. TGEV-infected pigs have antibodies directed against this site which cannot be found in PRCV-infected pigs (differential antibodies). These antibodies are demonstrated by a competitive blocking ELISA[3].

Even though TGEV and PRCV are antigenically so closely related, they have a different cell tropism. Whereas TGEV infects and destroys enterocytes on small intestinal villi, causing a malabsorption diarrhoea in pigs of all ages with a high mortality in neonatal piglets, PRCV infects epithelial cells in the respiratory tract causing a subclinical infection. In 1-week-old piglets, replication in a very few cells in the small intestine occurs[4]. On Belgian farms, pigs mostly become infected with PRCV between 5 and 10 weeks of age, while losing their maternal immunity. In pigs of this age, PRCV has been observed to replicate to high titre in the respiratory tract, whereas no replication was seen in small intestinal enterocytes[5].

The aim of the present study was to examine the effect of the PRCV-induced immunity on the duration of virus excretion and on the immune response following TGEV challenge.

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MATERIALS AND METHODS

Virus stocks

The TLM83 isolate of PRCV[1] and the TGEV strain Miller[6] were used in this study.

The TLM83 stock for experimental infection represented the first passage in swine testicle (ST) cells[7]. It was derived from a 20% suspension of lung tissue from an experimentally inoculated piglet. The infectivity titre of this stock was 10^7 tissue culture infective doses 50% ($TCID_{50}$) ml^{-1}. The pig infective doses 50% (PID_{50}) value of the stock was not determined.

The TGEV stock represented the third passage in hysterectomy-derived and colostrum-deprived 3-day-old piglets and was a 20% suspension of the small intestine. The infectivity titre in hysterectomy-derived and colostrum-deprived 3–7-day-old piglets was $10^{8.5}$ PID_{50} ml^{-1}.

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Pigs

The present study was performed on 22 TGEV-seronegative pigs and two pigs with maternal PRCV-antibodies. All pigs were conventionally reared and were of mixed breeding (Pietrain × Belgian Landrace). At the age of 4 to 6 weeks, the 24 pigs were divided into four groups which were housed separately.

Experimental groups

A first group of eight pigs was inoculated by aerosol with 10^7 $TCID_{50}$ TLM83 in 5 ml phosphate-buffered saline (PBS) (PRCV-AE group). The virus suspension was nebulized with the Wright nebulizer (particle size <8 μm) (Aerosol Products, Essex, UK).

A second group of four pigs was inoculated intragastrically with 10^7 $TCID_{50}$ TLM83 in 5 ml PBS on 2 subsequent days (PRCV-IG group). The inoculation was performed following laparotomy in order to avoid infection of the upper respiratory tract. The pigs were anaesthetized with 10 mg kg^{-1} methomidate (Hypnodil®; Janssen Pharmaceutica, Beerse, Belgium) and 2 mg kg^{-1} azaperone (Stresnil®; Janssen Pharmaceutica, Beerse, Belgium). Subsequently, the laparotomy was performed and the virus was injected through a catheter into the lumen of the stomach. The catheter was flushed twice with 5 ml PBS and drawn out of the stomach wall.

A third group of two seronegative pigs and two pigs with maternal PRCV antibodies was inoculated with TGEV into the gastric lumen via oral intubation (TGEV group). The pigs were first deprived of food for 24 h. Then, 62 ml of a 1.6% $NaHCO_3$ solution was administered intragastrically to neutralize gastric acidity. Fifteen minutes later, pigs were inoculated with 10^7 PID_{50} TGEV in 5 ml PBS. A preliminary experiment had shown that 10^7 PID_{50} TGEV strain Miller had to be inoculated to reproducibly cause an infection in 8–10-week-old seronegative conventional pigs.

A fourth group of eight seronegative pigs was not inoculated and served as a non-immune control group.

Samples taken before challenge

Blood was sampled from all pigs at the beginning of the experiment and at the moment of challenge for the demonstration of PRCV- and TGEV-specific antibodies.

Nasal swabs were collected from pigs of both groups inoculated with PRCV and from the non-immune control pigs. Rectal contents were sampled from pigs of both groups inoculated intragastrically with PRCV. The samples were collected daily for 14 days for viral isolation.

Challenge and samples taken following challenge

Four weeks after the start of the experiment, the pigs were housed together whereafter they were intragastrically challenged with 10^7 PID_{50} of TGEV in 5 ml PBS.

Six of the 24 challenged pigs were killed, namely a pig of the PRCV-AE group on days 2, 4 and 5 after challenge, a pig of the TGEV group on day 2 and a pig of the control group on days 2 and 5 (see *Table 2*). From these six pigs, faeces was sampled twice a day until they were killed. Immediately after bleeding the pigs, samples of the lungs (apical, cardial and diaphragmatic lobes), the trachea, the tonsils and the small intestine were collected for immunofluorescence. The small intestine was divided into seven segments, equal in length, designated duodenum, jejunum 1 to 5 and ileum. Three pieces ≈ 1.5 cm in length were collected. A piece of each lung lobe, tonsillar, tracheal and oesophageal swabs, the contents of each small intestinal segment and rectal contents were sampled for viral isolation. Rectal and ileal contents were also sampled for the detection of anti-TGEV-IgA antibodies. Blood was taken for the detection of PRCV- and TGEV-specific antibodies.

The 18 remaining pigs were monitored for the occurrence of diarrhoea. Furthermore, rectal contents were sampled twice a day for 14 days and subsequently twice a week until 4 weeks after challenge for viral isolation and the detection of anti-TGEV-IgA antibodies. Faeces were also sampled for bacteriological examination (enterotoxigenic *Escherichia coli*, *Salmonella*, *Clostridium*) from PRCV- and TGEV-immune pigs which developed diarrhoea after challenge. Blood was sampled weekly until 4 weeks after challenge for the detection of PRCV- and TGEV-specific antibodies and from pig number 2 also at 5 weeks after challenge for demonstration of TGEV-specific antibodies.

Viral isolation and immunofluorescence

All samples collected for viral isolation were immediately frozen at -70°C and kept at this temperature until processed. Viral isolation was performed using 20% suspensions (w/v) in PBS according to standard procedures. The supernatants from homogenates were inoculated into ten tubes fully sheeted with swine testicle cells 5 days after seeding the cells. The tubes have a diameter of 1.5 cm and contain 1 ml cell culture medium, namely Eagles minimal essential medium supplemented with 10% fetal calf serum, glutamine and antibiotics. The cell culture medium was supplemented with trypsin (5 μg ml^{-1}) if TGEV isolation was performed, but not for the isolation of PRCV. The cells were observed for the occurrence of cytopathogenic effect for 7 days. No second passage was performed.

Titrations of infectious TGEV in samples were performed by inoculating tenfold dilutions of the supernatants in microtitre plates on ST cells. The cell culture medium was supplemented with trypsin (5 μg ml^{-1}). Using trypsin in the medium increased the sensitivity of the titration tenfold. The high sensitivity of the titration method was shown by comparing the infectivity titre of the TGEV stock on ST cells with the titre in piglets. The titre was 10^9 $TCID_{50}$ ml^{-1} in cell culture compared with a titre of $10^{8.5}$ PID_{50} ml^{-1} in neonatal hysterectomy-derived and colostrum-deprived pigs at the age of 3 to 7 days.

Tissue pieces collected for immunofluorescence were mounted in a gelatin capsule with methocel (Fluka Chemie, Bornem, Belgium) and frozen in a liquid alcohol–carbon dioxide ice bath. Frozen sections (8 μm) were fixed in acetone before staining using the direct immunofluorescent method. A hyperimmune serum against TGEV, conjugated with fluorescein isothiocyanate, was used as a conjugate following standard procedures.

TGEV-neutralizing, TGEV-differential and anti-TGEV-IgA antibodies

All sera were tested for the presence of TGEV-neutralizing (VN) antibodies and the sera with VN

antibodies were further tested for the presence of TGEV-differential antibodies. Sera which did not contain the TGEV-differential antibodies, but contained VN antibodies, were considered to be directed against PRCV (PRCV antibodies). Sera which contained VN and TGEV-differential antibodies were considered to be directed against TGEV (TGEV-specific antibodies).

The virus neutralizing (VN) test for the detection of common TGEV- and PRCV-serum antibodies was performed in the swine kidney cell line SK6 using the Purdue 114 strain of TGEV[8]. This TGEV strain was used in the neutralization assay since it is highly adapted to the SK6 cells and since only one serotype of TGEV is known. Furthermore, it has been demonstrated that PRCV-induced antibodies neutralize TGEV and PRCV to the same titre[2]. The competitive inhibition ELISA for the detection of differential serum antibodies directed against TGEV[3] and the ELISA for the detection of TGEV-specific IgA in small intestinal and rectal content[9] were performed as previously described. All samples for IgA detection were diluted 1/2 in 0.5 M NaCl, pH 7.2 containing 0.05% Tween 80 and 10% fetal calf serum. Thirty-five samples from pigs not PRCV- or TGEV-infected were used to determine the limit between positive and negative absorbance values, namely ileal content collected via an ileal fistel from seven pigs, ileal content following humane killing from two pigs and faecal samples from 26 pigs. The samples showed absorbance values in the range 0.00–0.04. The mean absorbance of 0.02 plus three times the standard deviation resulted in absorbance values of 0.06. An absorbance of >0.1 was considered evidence of the presence of TGEV-IgA. Twofold dilutions of the sample were tested to determine the TGEV-IgA titre.

RESULTS

Virus excretion and immune response following inoculation

Following aerosol inoculation, PRCV was isolated from the nasal swabs for 6–11 days (mean: 8.5 days). Following intragastric inoculation with PRCV, no virus excretion could be demonstrated in the nasal secretions or the rectal contents. Following intragastric inoculation of TGEV, virus was isolated from the rectal contents of both seronegative pigs for 5 days, and from the rectal contents of the two pigs with maternal PRCV antibodies for 5 and 7 days, respectively. Virus was not isolated from the nasal swabs of the non-inoculated control pigs.

VN antibodies appeared in all the inoculated seronegative pigs. In the two pigs with maternal PRCV antibodies that became inoculated with TGEV, the mean VN titre decreased from 48 and 32 to 8 and 8, respectively, during the 4 week observation period. The non-inoculated pigs remained seronegative.

TGEV-differential antibodies were detected 4 weeks after the initial inoculation in the sera of the four TGEV-inoculated pigs and not in the sera of the PRCV-inoculated pigs or the non-inoculated pigs.

Clinical signs following challenge

The faeces became pasty to semiliquid in six out of eight pigs of the non-immune group, six out of eight pigs of the PRCV-AE group, in three out of four pigs of the PRCV-IG group and in one out of four pigs of the TGEV group during 4 to 6 days, 1 to 2 days, 1 to 3 days and 1 day, respectively.

TGEV excretion following challenge

The number of days during which TGEV was isolated from the faeces following challenge is given in *Table 1* for the 16 monitored pigs. In the PRCV-immune group, virus was either not isolated from the faecal samples, as occurred in three out of five of the monitored (pig nos 2 to 4) and one out of three of the killed pigs (pig no. 8) of the PRCV-AE group, or was isolated during a shorter period, namely 2 to 4 days (pig nos 1, 5 to 7 and 9 to 12), than from faeces of the seronegative pigs (5–6 days). TGEV was not isolated from the faecal samples of the TGEV-immune pigs.

The mean TGEV titre in the faecal samples of the seronegative pigs was 10^2 $TCID_{50}$ TGEV per gram faeces (range $10^{1.4}$–$10^{2.4}$ $TCID_{50}$) on day 2 after challenge and it remained similar until day 6 after challenge when it still was $10^{1.8}$ $TCID_{50}$ TGEV per gram faeces (range $10^{1.6}$ to $10^{1.9}$ $TCID_{50}$). These titres were not significantly different from the titres in the faecal samples of the pigs of both PRCV-immune groups.

There was no correlation between the occurrence of virus excretion and of diarrhoea. Two pigs of the non-immune group and one of the PRCV-IG group showed virus excretion in the absence of diarrhoea and two pigs of the PRCV-AE group and one of the TGEV-immune group showed pasty faeces for 1 to 2 days even though no virus could be isolated from the faeces. Enteropathogenic bacteria could not be demonstrated in the faecal samples.

Immunofluorescence and viral isolation on samples of the killed pigs

The results of the immunofluorescence on the seven small intestinal segments and of the viral isolation on the small intestinal contents of the killed pigs are presented in *Table 2*.

Fluorescence was observed in epithelial cells on villi of the seronegative pigs slaughtered 2 and 5 days after challenge and of the PRCV-immune pig slaughtered 2 days after challenge. The extent of fluorescence did not differ significantly between these three pigs. The percentage of villi with fluorescing cells was low or negative in the duodenum, increased in the mid jejunum to 50–100% and was lower again in the ileum. Maximally 25% of the epithelial cells on a villus and mostly only one to five cells were fluorescing.

Fluorescence was not observed in the small intestinal mucosa of the PRCV-immune pigs killed 4 and 5 days after challenge and of the TGEV-immune pig killed 2 days after challenge. In both PRCV-immune pigs, virus was not isolated from the small intestinal contents. In the TGEV-immune pig, however, virus was isolated in low titres from the contents of cranial small intestinal segments and from the oesophageal swab.

Virus replication was not seen in lungs, trachea and tonsils by immunofluorescence and virus was not isolated from lung lobes and from tracheal and tonsillar swabs.

Immune response following challenge

The immune response following challenge of the pigs with TGEV is presented in *Table 1*. In all the seronegative

Table 1 Immune response and duration of virus excretion after TGEV challenge of pigs immune following infection with PRCV or TGEV or of non-immune pigs

Group	Pig number	Serum antibodies: Virus neutralization titre WPC[a]	0	1	2	3	4	TGEV differential[b] 0	4	5	Faeces: TGEV IGA DPC[a] From	To	Days TGEV isolation
PRCV aerosol	1		96	256	512	1536	960	–	+	ND[d]	5	17	3
	2		128	96	64	192	192	–	–	+	Not detected		0
	3		64	768	256	256	92	–	+	ND	15	17	0
	4		32	128	32	32	24	–	+	ND	12	14	0
	5		48	960	1280	960	640	–	+	ND	7	10	4
PRCV intragastric	9		8	1280	1536	4096	4096	–	+	ND	Not detected		4
	10		32	4096	2048	4096	4096	–	+	ND	Not detected		4
	11		48	960	1920	640	1280	–	+	ND	Not detected		2
	12		6	960	640	256	384	–	+	ND	Not detected		4
TGEV intragastric	13		64	48	96	64	96	+	+	ND	Not detected		0
	14		48	24	48	24	48	+	+	ND	Not detected		0
	15[c]		8	12	8	4	8	+	+	ND	Not detected		0
Non-immune control	17		<2	4	24	64	96	–	+	ND	Not detected		6
	18		<2	<2	12	16	64	–	+	ND	Not detected		6
	19		<2	<2	48	192	256	–	+	ND	Not detected		6
	20		<2	4	24	256	128	–	+	ND	Not detected		5
	21		<2	<2	16	16	48	–	+	ND	ND[d]		5
	22		<2	32	96	192	96	–	+	ND	ND		6

[a]WPC: weeks after challenge; DPC: days after challenge
[b]– = titre <5; + = titre ⩾ 5
[c]Maternal PRCV antibodies at the moment of the first TGEV inoculation
[d]ND, not determined

Table 2 Virus replication in the small intestine of PRCV-immune, TGEV-immune or non-immune pigs challenged with TGEV and slaughtered at different times after inoculation

Groups	Pig number	Slaughter days after challenge	Virus neutralization in serum: At challenge	At slaughter	Immunofluorescence score[a]: Duodenum	Jejunum 1	Jejunum 2	Jejunum 3	Jejunum 4	Jejunum 5	Ileum	Small intestinal contents: TGEV isolation[b]	TGEV IgA titre
PRCV aerosol	6	2	96	32	+	+ +	+	+ – +	+ + +	+ + + – +	+	ND	<2
	7	4	256	384								Negative	32
	8	5	24	64			–			–		Negative	2
TGEV	16[c]	2	6	12		–					–	Positive	4
Control	23	2	<2	<2	+	+	+	+ +	+ + +	+	+	ND	<2
	24	5	<2	<2	–	–	+	+	+ + + +	+ + + +	+	ND	<2

[a] = no immunofluorescence; + ⩽25 per cent villi with fluorescing cells; + + 26 50%; + + + 51 75%; + + + + >75%
[b]ND = not determined; negative = no virus isolated (virus titre $<10^{1.3}$ $TCID_{50}$ g^{-1}); positive = $10^{1.8}$ $TCID_{50}$ g^{-1}
[c]Maternal PRCV-antibodies at the moment of the first TGEV inoculation

pigs, VN antibodies appeared 1 to 2 weeks after challenge. The titres gradually increased until 3 to 4 weeks after challenge. At that time, titres were between 64 and 256. In the TGEV-immune pigs, the challenge did not induce an increase in the VN titre. The titres remained between 8 to 96 during the 4 week observation period. In all except one pig (pig no. 2) of both PRCV-immune groups, the challenge resulted in a rapid rise in the VN titre. The titre reached a peak 1–3 weeks after challenge. The peak was higher in most pigs of the PRCV-IG group (VN titres 960–4096) than in the PRCV-AE group (VN titres 128–1536). Furthermore, the secondary immune response was more pronounced in the PRCV-immune pigs which excreted TGEV (peak VN titres 960–4096) than in those which did not (peak VN titres 128–768).

TGEV-differential serum antibodies appeared 4 weeks after challenge in all the pigs of the non-immune and in all pigs of PRCV-immune groups except pig number 2. In the latter pig, the TGEV-specific antibodies appeared 1 week later.

TGEV-specific IgA was not detected in the faeces of the pigs at the moment of challenge. Following challenge, TGEV-specific IgA appeared in low titres (namely 2 to 8) in the faeces of the four pigs of the PRCV-AE group which seroconverted and not in the faeces of the pig that did not seroconvert or in the faeces of the pigs of the other groups. TGEV-specific IgA was detected in all four pigs during the second week after challenge.

TGEV-specific IgA was also detected in the ileal contents of the two pigs of the PRCV-AE group which were killed 4 and 5 days after challenge, respectively, and in the pig of the TGEV group killed 2 days after challenge (*Table 2*). Furthermore, TGEV-IgA was detected in the rectal contents of the PRCV-immune pig killed 4 days after challenge. It was not detected in the intestinal or rectal contents of any of the other killed pigs.

DISCUSSION

The inoculation of 8–10-week-old pigs with 10^7 PID_{50} TGEV induced a reproducible infection as evidenced by the excretion of infectious TGEV for 5–6 days in all

monitored pigs of the non-immune control group. The infection, however, did not consistently induce diarrhoea or induced only a mild diarrhoea. TGEV always induces severe diarrhoea in neonatal piglets whereas a subclinical infection or mild disease is observed in growing pigs[10]. The increased resistance to disease in growing pigs has been attributed to a more rapid replacement of the villous epithelium[11] and to an increased capacity of the colon to absorb water in comparison with both mechanisms in neonatal piglets[12]. The fact that a TGEV-immune pig developed a pasty faeces following TGEV challenge even though no virus or enteropathogenic bacteria could be isolated from the faeces indicates that not only the TGEV infection but also other factors such as possibly deprivation of food prior to the challenge and stress due to the manipulation at challenge could be partly responsible for the increased faecal fluid content.

The TGEV-immune pigs were completely protected against TGEV challenge. Indeed, no TGEV excretion in faeces or virus replication in villous enterocytes was observed and no serological response occurred after the challenge. The virus which was isolated from the oesophageal swab and from contents of the cranial intestinal segments in the pig killed 2 days after challenge must have been swallowed out of the environment since virus was not demonstrated in the respiratory tract and tonsils by immunofluorescence and/or virus isolation in this pig or in any of the other killed pigs.

None of the monitored PRCV-immune pigs were completely protected against challenge as evidenced by the appearance of TGEV-specific serum antibodies 4 to 5 weeks after challenge in all and a rapid secondary humoral immune response in most PRCV-immune pigs. This is in agreement with previous results obtained by van Nieuwstadt *et al.*[13]. Nevertheless, the degree of virus replication must have been limited in four of eight pigs immune following aerosol inoculation with PRCV since no TGEV was isolated from the faeces of these pigs. This suggests a partial protection against the TGEV infection in these pigs. Furthermore, all other PRCV-immune pigs showed a decrease in the duration of excretion of infectious TGEV in comparison with the duration of virus excretion by seronegative pigs. This suggested that the PRCV-immune pigs eliminated the TGEV infection more rapidly, or that they produced lower quantities of infectious virus from day 4–6 than the seronegative pigs. Low quantities of infectious virus produced in the small intestine are not detected in the faeces by viral isolation (unpublished observation) indicating that inactivation of virus may occur during the passage in the large intestine. Results in the killed pigs showed that at least some PRCV-immune pigs eliminated the infection more rapidly than the seronegative pigs, since virus replication could not be demonstrated in the small intestine of the PRCV-immune pigs killed on days 4 and 5 after challenge, whereas replication was seen in the seronegative pig killed on day 5 after challenge.

Van Nieuwstadt *et al.*[13,14] could not demonstrate a decrease in the duration of virus excretion upon TGEV challenge of PRCV-immune pigs. The difference between their results and the results in the present study cannot be explained. It should be mentioned that van Nieuwstadt *et al.*[13,14] performed their experiments in specific pathogen-free (SPF) pigs. It has been observed in 8–10-week-old mice, that SPF mice have significantly less IgA-secreting plasma cells in the small intestinal lamina propria and in mesenteric lymph nodes than conventionally reared mice[15]. It was not examined whether this difference influenced the immune response. Furthermore, van Nieuwstadt *et al.* used ELISA to demonstrate TGEV excretion. ELISA detects not only infectious virus but also inactivated virus. It should be determined if viral antigen can be detected in the faeces for a longer period than infectious TGEV.

In the present study, results were obtained which suggest that the decreased duration of TGEV excretion was due to a local secondary immune response occurring at the intestinal mucosa. TGEV-IgA was detected in the faeces after TGEV challenge of pigs immune following aerosol inoculation with PRCV. TGEV-IgA could not be detected in the rectal contents of the four TGEV-immune pigs, but could be detected in low titre in the intestinal contents of the TGEV-immune pig slaughtered 2 days after challenge. Most probably, high TGEV-IgA titres have to be secreted at the intestinal mucosa before IgA can be detected in the faeces as seen in the PRCV-immune pig killed 4 days after challenge. Therefore, the appearance of IgA in the faeces sampled 1–2 weeks after challenge suggests that a secondary immune response occurred at the intestinal mucosa. This is in agreement with the observation that an IgA booster response occurs in the milk of PRCV-immune sows which become infected with TGEV. The sows obtain very high TGEV-IgA titres (>1024–$10\,240$) in their milk within 1 to 2 weeks after the TGEV infection (Pensaert and Hooyberghs, unpublished results). It is well known that the antigenic stimulation of most of the IgA-secreting plasma cells in mammary tissue occurs in the gut-associated lymphoid tissue (GALT). The stimulated cells migrate to the mammary gland shortly before parturition and during lactation. There they secrete IgA in the milk. This phenomenon is known as the gut–mammary immunologic link[16]. The presence of an IgA booster response in milk and in faeces after TGEV challenge, indicates that PRCV-primed IgA-positive cells appear in the intestinal mucosa following an infection with PRCV.

Results in the present study show that priming of the immune system can occur by infectious PRCV that reaches the intestinal tract since pigs, intragastrically inoculated with PRCV, developed VN antibodies in the absence of a respiratory infection and showed a decreased duration of TGEV excretion and a secondary immune response following the challenge. It has been demonstrated that small amounts of infectious virus produced in the respiratory tract become ingested and reach the small intestine[5]. Replication of PRCV, however, could not be demonstrated in small intestinal epithelial cells of 5–6-week-old seronegative pigs which were inoculated directly into the lumen of the cranial jejunum with 10^5 $TCID_{50}$ PRCV[5]. In the present study, intragastric inoculation with a higher dose, namely 10^7 $TCID_{50}$ of PRCV twice within 24 h, induced virus-neutralizing antibodies. An attempt was made to induce virus-neutralizing antibodies in two seronegative pigs by oral administration of 10^7 $TCID_{50}$ UV-inactivated PRCV, twice a day for 14 days, without success (unpublished results). The fact that neutralizing antibodies appeared following the intragastric inoculation with PRCV, therefore, indicated that intestinal replication of PRCV occurred.

It remains to be determined whether intestinal

replication occurs in pigs with a respiratory tract infection, even though there is evidence of priming of the intestinal mucosal immune system in these pigs. Indeed, priming of the intestinal mucosal immune system can be induced not only by a direct antigenic stimulation of the GALT as a result of an intestinal infection but likely also by migration of stimulated IgA-lymphocytes from the bronchus-associated lymphoid tissue to the gut mucosa[17].

In conclusion, an infection of pigs with PRCV primes the systemic and mucosal humoral immune system against TGEV, so that a subsequent challenge with TGEV results in a secondary antibody response and in a decreased duration of excretion of infectious TGEV. In the field, pigs frequently become infected with PRCV around the age of 5 to 6 weeks in the presence of declining maternal antibody titres. It remains to be determined if these pigs also show a decreased duration of virus excretion following TGEV challenge.

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