SITES OF REPLICATION OF A PORCINE RESPIRATORY CORONAVIRUS IN 5-WEEK-OLD PIGS WITH OR WITHOUT MATERNAL ANTIBODIES

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ABSTRACT

On farms, where the porcine respiratory coronavirus (PRCV) is enzootic, pigs usually become infected between 5 and 10 weeks of age while losing their maternal antibodies. It was examined whether PRCV replicates in the small intestine in such pigs. This point is important since intestinal replication with PRCV might induce immunity against TGEV not only by stimulating mucosal intestinal immunity, but also by the induction of a lactogenic IgA response at later age via the gut-mammary link.

Five week old pigs with and without maternal antibodies were inoculated by aerosol or directly into the intestinal lumen. In aerosol inoculated pigs, virus replication was observed to high titres in the respiratory tract. Replication occurred in epithelial cells of nasal mucosa, trachea, bronchi bronchioli and alveoli and in alveolar macrophages. Small amounts of virus produced in the respiratory tract were ingested, but no intestinal replication of PRCV was demonstrated. Differences were not observed in virus titre and sites of replication in seronegative pigs compared to those in pigs with maternal antibodies. Upon inoculation of 10^5 or 10^7 TCID₅₀ directly into the lumen of the cranial jejunum, no intestinal replication could be demonstrated.

INTRODUCTION

In 1984, a porcine respiratory coronavirus (PRCV) appeared in the swine population of Belgium (1). This virus was antigenically closely related to the enteropathogenic coronavirus, transmissible gastroenteritis virus (TGEV) (2). Upon infection with PRCV, pigs develop TGEV-neutralizing antibodies. PRCV has spread to such an extent that nearly 100 % of the Belgian swine farms are now infected (3). Similar spread has been observed in other European countries (4,5,6).

Presently, the virus is enzootic in the Belgian swine population. Regular episodes of infection and reinfection occur on breeding farms. On these farms, pigs usually become infected between 5 and 10 weeks of age while losing their maternal antibodies (3). The aim of the present study was to examine if PRCV replicates in the intestine of such pigs. This point is important since intestinal replication of PRCV may result in the induction of immunity against TGEV by stimulating IgA secreting lymphocytes in the gut-associated-lymphoid-tissue (GALT). In this way, a PRCV infection may not only induce an intestinal mucosal immunity against TGEV, but also an anti-TGEV lactogenic IgA response at a later age via the gut-mammary-link (7), as observed following TGEV infection (8).

MATERIALS AND METHODS

Virus stock

The TLM 83 isolate of PRCV was used in this study (1). The stock for the experimental infection represented the first passage in ST cells of a 20 percent suspension of lung tissue from an experimentally inoculated pig. The infectivity titer of this stock was $10^{6.7}$ (TCID₅₀) per ml.

Inoculation by Aerosol of 5-week-old Pigs with or without Maternal Antibodies

Three colostrum-deprived pigs and three conventionally reared pigs with maternal PRCV antibodies were used in this experiment. Serum was sampled at inoculation. The pigs were inoculated by aerosol with $10^{7.0}$ TCID₅₀ TLM 83 in 8 ml phosphate buffered saline (PBS) at the age of 5 weeks. The virus suspension was nebulized using the "Wright" nebuliser (particle size <8 µm) during 40 minutes for each pig individually. Rectal swabs were collected daily for viral isolation (VI). One seronegative and one seropositive pig were killed at 3, 4 and 5 days post inoculation (PI) respectively. Immediately after bleeding the pigs, the following tissues were collected for VI and immunofluorescence (IF): nasal mucosa, trachea, lungs (apical, cardiac, diaphragmatic lobes), tonsils, small intestine, spleen and lymph nodes (mesenteric, bronchial). The small intestine was divided in 7 segments of equal length: duodenum, jejunum 1 to 5 and ileum. A piece of approximately 1.5 cm long was removed for IF. VI was performed on the remaining parts. Caecal, rectal contents and stomach tissue were collected for VI only.

Furthermore, a post mortem lung washing was performed by injecting 50 ml of PBS containing 50 percent (v/v) Alsever's solution through a polyethylene catheter in the left bronchus. The fluid was immediately aspirated whereafter the cells were sedimented by centrifugation (10 minutes at 300 g). Next, the erythrocytes were lysed with NH₄Cl. Sedimented cells were resuspended in 5 ml Eagle's minimum essential medium, whereafter smears were prepared for IF.

Some additional samples were collected for VI in the seronegative pigs only i.e. plasma daily between the time of inoculation and euthanasia; small intestinal contents, liver, plasma and inguinal and cervical lymph nodes at euthanasia. In seropositive piglets, the following samples were also collected for VI at euthanasia: oesophageal swabs and contents of stomach and of the 7 small intestinal segments.

Intraintestinal Inoculation of 4- to 5-week-old Pigs with or without Maternal Antibodies

Three conventionally reared pigs without seroneutralizing antibodies were housed together in isolation. They were inoculated with PRCV directly into the lumen of the cranial jejunum after laparotomy at the age of 4 weeks. The inoculation dose was 10^5 TCID₅₀ TLM 83 in 5 ml PBS. They were followed serologically during 4 weeks. Rectal and tonsillar swabs were collected for VI daily until 2 weeks PI, whereafter they were collected 3 times a week until 4 weeks PI.

A catheter was implanted after laparotomy in the ileum of a 5-week-old pig with maternal TGEV antibodies in order to allow frequent sampling of intestinal content at the site of virus replication. PRCV was inoculated in a dose of 10^7 TCID₅₀ TLM 83 in 5 ml PBS into the lumen of the cranial jejunum at the time of surgery. Serum was sampled at inoculation and 3 weeks later. Ileal contents were collected for VI at 4 hours interval during the first 3 days PI and subsequently 2 times a day until 1 week PI.

Viral Isolation, Viral Titration, Immunofluorescence and TGEV sero-neutralization test

VI isolation was performed according to standard procedures. The supernatant of a 20 percent suspension in PBS of each sample was inoculated on swine testicle (ST) cells in Flow tubes. Quantitative titrations of infectious virus were performed by inoculating 10-fold dilutions of the supernatants on ST cells in microtiter plates. The IF-test was performed using as hyperimmune serum a specific antiserum to TGEV. The SN-test was performed in SK6 cells using the Purdue-114 strain of TGEV.

RESULTS

Sites of Replication of PRCV upon Aerosol Inoculation of 5-week-old Pigs with or without Maternal Antibodies

No virus was isolated from rectal swabs sampled daily. Virus was isolated from one plasma sample of the seronegative pig 1665 taken one day PI.

Results of virus titration and of IF of tissues of 3 pigs without and 3 with maternal antibodies inoculated by aerosol and euthanatized 3, 4 and 5 days PI are presented in Table 1. Virus was isolated from nasal mucosa, trachea, lungs, tonsils and bronchial lymph nodes of all 6 pigs. The highest titres were detected in lung tissue (up to $10^{6.3}$ in seronegative and up to $10^{7.3}$ TCID₅₀ per g tissue in pigs with maternal antibodies). Titres decreased in seronegative pigs in most respiratory tract tissues and in bronchial lymph nodes from 3 up to 5 days PI. Fluorescence was observed in all the pigs in the epithelial cells of nasal mucosa, trachea and lungs. IF in lungs was seen in epithelial cells of alveoli, bronchioli and rarely of bronchi. Furthermore, fluorescence was observed in alveolar macrophages in the lung washing fluids in all pigs.

Table 1. Results of virus titration and immunofluorescence of tissues from pigs with and without maternal antibodies inoculated by aerosol with TLM 83 at the age of 5 weeks

	Virus titre (log ₁₀ TCID ₅₀ /g in pig number)						IF ^a
	1665 ⁿ	1845 ^{ma}	1666 ⁿ	1847 ^{ma}	1667 ⁿ	1846 ^{ma}	all pigs
Day killed p.i.	3	3	4	4	5	5	3, 4, 5
<u>Tissue</u>							
<u>Respiratory tract</u>							
Nasal mucosa	4.5	5.7	4.5	5.5	4.7	5.0	+
Trachea	4.5	5.0	4.3	4.3	3.9	4.0	+
Lung	6.3	7.3	6.0	6.5	4.0	6.7	+
Tonsil	2.1	4.5	1.8	3.3	1.3	4.5	-
Bronchial lymph nodes	3.7	4.5	2.2	3.3	1.8	4.5	-
<u>Digestive tract</u>							
Oesophageal swab	nt ^b	2.7	nt	2.7	nt	1.8	
Stomach	neg. ^c	2.2	1.4	neg.	neg.	neg.	nt
Duodenum	1.5	neg.	1.3	neg.	neg.	neg.	-
Jejunum 1	neg.	neg.	1.3	neg.	neg.	neg.	-
Jejunum 2-5	neg.	neg.	neg.	neg.	neg.	neg.	-
Ileum	neg.	neg.	neg.	neg.	neg.	neg.	-
Caecal contents	neg.	neg.	neg.	neg.	neg.	neg.	-
Mesenteric lymph nodes	neg.	neg.	1.7	neg.	neg.	neg.	-
<u>Other</u>							
Liver	neg.	nt	neg.	nt	neg.	nt	nt
Peripheral lymph nodes ^d	neg.	nt	neg.	nt	neg.	nt	nt
Spleen	neg.	nt	neg.	nt	neg.	nt	nt
Plasma	neg.	nt	neg.	nt	neg.	nt	

a: -, no fluorescence; +, 5% or less of epithelial cells fluorescing. b: nt, not tested.

c: neg, no virus isolated. d: inguinal and cervical lymph nodes. n: no maternal antibody. ma: maternal antibody titre of 48

Virus was isolated from the stomach, gastric content, the mesenterial lymph nodes and the cranial small intestine in some pigs and from oesophagal swabs in all the seropositive pigs. Fluorescence was not seen in any of the pigs in the small intestine or mesenterial lymph nodes.

Intraintestinal Inoculation of 4- to 5-week-old Pigs with or without Maternal Antibodies

A TGEV-SN-titer of 6 appeared 4 weeks PI in 1 of 3 seronegative pigs which had been inoculated into the lumen of the cranial jejunum. No virus was isolated from rectal swabs of this pig taken between 1 to 7 days PI. The other 2 pigs remained seronegative. Upon the intraintestinal inoculation, virus was not isolated from any sample of ileal content collected during the 1st week PI in the pig with the ileal catheter. The neutralizing-titer in the serum of the latter pig was 8 at inoculation and 3, 3 weeks later.

DISCUSSION AND CONCLUSION

No significant differences were observed in virus titres and immuno-fluorescence in respiratory tract tissue between the 5-week-old pigs with or without maternal antibodies. These results show that low maternal antibody titres do not decrease virus production at the respiratory tract surface. A similar observation was made upon infection with bovine respiratory syncytial virus of calves with or without maternal antibodies (9). In this latter study, it was suggested that the results could be attributed to the presence of only a weak diffusion of serum antibodies into lung alveoli.

Previous pathogenesis experiments have been performed in which 1-week-old piglets were inoculated with PRCV by aerosol (10). The virus was observed to replicate at high titres in the respiratory tract (up to $10^{8.3}$ TCID₅₀ TLM 83 per g lung tissue). Replication occurred in epithelial cells of the nasal mucosa, trachea, bronchi, bronchioli and alveoli and in alveolar macrophages. Replication was also observed in tonsillary tissue. In the present study, replication of PRCV in tonsils could not be demonstrated by fluorescence. Virus titres and fluorescence observed in lung tissue of the 5-week-old pigs were generally lower than those in the 1-week-old piglets.

Upon aerosol inoculation, virus was isolated inconsistently and at low titres from the cranial digestive tract in pigs with or without maternal antibodies. Since no fluorescence was found in the cranial small intestine, this was considered to be ingested virus.

In 1-week-old piglets, PRCV was observed to replicate in the small intestine upon aerosol inoculation (10). The intestinal replication of PRCV started in the ileum and spread within a few days to the duodenum. Intestinal replication remained limited to few unidentified cells. In the present study, virus could not be isolated either from mid and caudal small intestine or from contents of this part of the small intestine.

It was previously shown that the susceptibility of the small intestine of 1-week-old piglets to infection with PRCV is very low. More than 10^3 TCID₅₀ of TLM 83 must be inoculated into the lumen of the cranial jejunum in order to induce an intestinal infection consistently (11). Results of the present study show that the small intestine in older pigs is fully resistant to PRCV infection since inoculation of 10^5 to 10^7 TCID₅₀ into the lumen of the cranial jejunum induced no intestinal replication. The very weak and late serological reaction observed in one seronegative pig may have been due to gastrointestinal antigen uptake (12).

The results of the present study show that no intestinal replication occurs upon aerosol inoculation with PRCV of older pigs. Therefore, TGEV- specific IgA which was observed in the milk of sows previously infected with PRCV (Callebaut *et al.*, this volume) is most likely induced by stimulating lymphocytes present in the bronchus-associated-lymphoid-tissue. The occurrence of TGEV-IgA in milk following respiratory PRCV infection suggest the presence of a common mucosal immune system and a lung-mammary link in swine.

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