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Disorders Induced by the Experimental Infection of Pigs with the Porcine Respiratory Coronavirus (P. R. C. V.)

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With 2 tables

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Summary

Five colostrum deprived hysterectomy-derived piglets were inoculated by the intratracheal route with a Porcine Respiratory Coronavirus (PRCV). The clinical effects and growth performances were compared with those in 5 similar piglets which were inoculated with Minimum Eagle Medium only. A transient hyperthermia, mild to severe dyspnoea, polypnoea and an obvious cessation of growth were recorded on infected pigs only whereas the control group did not show any adverse reaction after inoculation. The reasons for the variability of effects of experimental infection observed in previous trials, are discussed.

Introduction

A porcine respiratory, non enteric coronavirus (PRCV) related to Transmissible Gastroenteritis virus (TGE) has been isolated in different countries of Europe since 1986 (1, 2, 8). It has become very widespread among the swine populations in Belgium, Denmark (4), France, Great-Britain and other European countries but its pathogenicity has not been well defined. No particular clinical sign was observed after an experimental infection, by the oronasal route, into young piglets (8). However, mild respiratory disorders in fattening pigs have been associated with the natural infection on the field (3, 5). Intratracheal inoculation has been used with the pig influenza viruses (6) and with PRCV (2). This paper describes further experimental infections of PRCV using intratracheal inoculation.

Material and Methods

Pigs

Ten Belgian Landrace × Pietrain specific pathogen free pigs obtained by hysterectomy were divided into 2 groups, at 90 days-of-age (average weight 38 kg). The first group of five pigs was infected with a PRCV strain. The other group from the same origin, was inoculated in similar conditions but with Minimum Eagle Medium (MEM) only. Each group was kept totally isolated from the other one.

Challenge procedure

The strain isolated in France (2) was used. In a previous experiment (7), nasal swabs were taken from infected SPF pigs. After mixing the cotton swab in 2 ml of MEM, 1 ml was inoculated on a Swine

Testis (ST) cell line. A cytopathogenic effect was noted 3 days after inoculation. This cytopathogenic effect was neutralized with a monospecific hyperimmune TGE serum. The cells and medium were frozen. After thawing and slow centrifugation the titre of the supernatant was determined. The titre on ST cells was $10^{5.7}$ Tissue Culture Infectious Dose $(TCID)_{50}$/ml. Fifteen ml of the viral suspension were mixed with 40 ml of MEM. Each pig in the infected group received intratracheally 10 ml of the suspension mixture. For this purpose, the pigs were estrained using a saubbing rope round the upper jaw, in the standing position. The lower jaw was also fixed in position with a small rope fastened onto the floor. By raising the upper jaw, the mouth was maintained opened and, most of the time, the glottis was easily visible. A sterile human urinary cannula (sonde vesicale droite ORX — L 40 cm Vygon, Réf. 420-12) was inserted approximately 30 cm into the trachea. Then, when the pig inhaled, the 10 ml of viral suspension mixture was inoculated through the cannula.

Each control pig was inoculated with 10 ml of MEM without virus using exactly the same procedure.

Clinical observations, performance assessment

Clinical signs and, in particular, respiratory symptoms and rectal temperature were recorded daily. Each pig was weighed at 14 and 7 days prior to the experimental infection (Do). Subsequently, they were weighed at 4, 7, 14, 21 days after infection. Weight losses following challenge and the duration of hyperthermia were the main criteria for evaluating the challenge effects.

Serology

Transmissible Gastroenteritis virus neutralizing antibodies were looked for in the pig sera using the technique described by TOMA and BENET, 1976. Antibodies titres were expressed as the value of the inverse of the highest serum dilution neutralizing $100\,TCD_{50}$ of the Purdue strain of TGE virus.

Necropsy

Three weeks after the experimental infection, all the pigs were slaughtered and necropsied. Particular attention was given to lung lesions.

Statistical tests

The unilateral COLIN-WHITE test and the student test were used to compare performances of the 2 groups.

Results

Clinical observations

Clinical signs were absent in control pigs. Hyperthermia ($40°3 < \text{temp.} < 40°9$ C) appeared in 3 infected pigs, 24 hours after challenge. 48 hours after challenge, temperature was normal in the infected group (mean 39°2 C standard deviation : 0.3). The same 3 pigs showed also dyspnea, polypnea and prostration. The 2 others remained clinically normal.

Assessment of performance

Table 1 shows the differences of performances between the 2 groups. Before challenge (Do), the performances were similar. After challenge the control pigs grew faster than infected pigs between day 0 and day 4 and between day 0 and day 21 ($p < 0.05$) (Table 1). Between day 0 and day 7, the difference between the 2 groups is discernible but is not statistically significant. It has to be emphasized that in the infected group, the performance of 1 of the 5 pigs was practically not affected by the challenge.

Table 2 shows the neutralizing antibody titres obtained against the TGE Purdue strain. A clear seroconversion was observed in infected pigs whereas no antibody appeared in the control group.

Necropsy

Limited lesions from 1 to 3 cm^2 of pneumonia were seen on lungs of 2 infected pigs. These lesions were associated with ancient scarred grooves in the lung parenchyma. These scarred grooves were also observed in 2 other pig lungs of the infected group. Histological studies of macroscopic pneumonia lesions indicated an interstitial pneumonia with atelec-

Table 1. Analysis of mean growth rate (standard deviation) in the 2 groups of pigs (infected and control)

Group	Total weight loss or gain (kg)			Mean daily gain (g)			
	D_{-31}–D_0	D_0–D_4*	D_0–D_7	D_{-31}–D_0	D_0–D_4	D_0–D_7	D_0–D_{21}
Infected	+18.6	−0.4	+2.4	600 (195.7)	−100** (379.1)	342.9 (313)	542.9** (133)
Control	+19.4	+2.6	+4.7	625.8 (48.9)	650** (136.9)	671.4 (119.5)	771.4** (85.2)

* Period in days between day of challenge (D_0) and 4 days later (D_4). ** Difference is statistically significant ($P < 0.05$).

tasis, oedema and thickened alveolar walls, and a slight peribronchial accumulation of lymphocytes and macrophages. Fibrinous exudate was seen in the lumen of some alveoli. Gross lesions were absent from the lungs of control pigs.

Discussion

In this experiment, in relation with PRCV were induced slight clinical signs. The effects of the PRCV on pigs were measured objectively by the evaluation of growth rates in the infected and control groups. In spite of the low number of pigs in each group, the differences in growth rate were statistically significant for the first 4 days after inoculation; resulting is a significant difference over the first 21 days after inoculation. It has to be emphasized that, in the infected group, one pig showed no clinical signs and its growth performances were not affected by the experimental infection.

Failure to insert the cannula for enough into the trachea may explain the absence of effects of challenge in this pig.

These results differ from those obtained by PENSAERT et al. (8), but are quite similar to the ones observed by DURET et al. (2). In the former case, colostrum-deprived hysterectomy-derived piglet were inoculated by the intranasal route, which could explain this discrepancy in results. In the latter case, the induced clinical signs seem to be more severe than the ones shown in the present study. The health status of the infected pigs could explain the variability in the intensity of the effects of the experimental infection. Indeed, in the present study, this health status of pigs was very high as all of them had been obtained by hysterectomy and were artificially suckled. It has been shown that, with other viruses with a respiratory tropism, severity of the signs varies according to the original health status of the infected pigs (10). Such an observation could also explain the variability of the infection of pig herds on the field.

Table 2. TGE neutralizing antibody titres detected in the pig sera of 2 groups (infected and control)

Group	Pigs n°	D_0*	D_7	D_{14}
Infected	1	<0	<0	+ 64
	2	<0	<0	+ 64
	3	<0	+2	+ 32
	4	<0	<0	+ 32
	5	<0	<0	+128
Control	6	<0	<0	<0
	7	<0	<0	<0
	8	<0	<0	<0
	9	<0	<0	<0
	10	<0	<0	<0

* Challenge day.

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Zusammenfassung

Störungen bei Schweinen nach experimenteller Infektion des Atmungstraktes mit einem Coronavirus des Schweines

Nach Geburt durch Hysterektomie wurden fünf Ferkel, denen Kolostrum vorenthalten wurde, mit PRCV intratracheal inokuliert. Fünf ähnliche Ferkel, die nur mit Minimum Eagle Medium inokuliert wurden, dienten als eine Kontrollgruppe. Klinische Erscheinungen sowie Wachstum bei den zwei Gruppen wurden verglichen.

Vorübergehende Hyperthermie, milde bis schwere Dyspnoe, Polypnoe sowie eine auffallende Wachstumshemmung konnten nur bei infizierten Ferkeln beobachtet werden, wobei die Kontrollgruppe keinerlei ungünstige Folgen der Inokulation mit Medium aufwies. Die Gründe für die unterschiedlichen Ergebnisse früherer Versuche mit experimenteller Infektion werden diskutiert.

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