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Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion

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ABSTRACT

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Twelve pigs were experimentally infected with a porcine respiratory coronavirus (PRCV) by the oronasal route. Viral excretion was measured daily by two means–deep nasal swabs and air samples obtained in a cyclone sampler. Clinical signs were very slight on infected pigs. Airborne virus could be recovered from day 1 to day 6 post-infection in the cyclone sampler as well as in petri dishes placed in the same loose-box. Viral titres obtained from nasal swabs were significantly correlated with those obtained from air samples. Different collection media were compared. The most efficient media for the collection of infectious viral particles contained a protective agent such as foetal calf serum.

INTRODUCTION

Respiratory disorders due to viruses are the main problems recorded in areas of intensive porcine production. Common causes of the disease includes pseudorabies virus (PRV), influenza viruses and the porcine respiratory coronavirus (PRCV), the latter being first detected in different European countries in 1983–1984 (Brown et al., 1986; Duret et al., 1988; Pensaert et al., 1986). Serological studies have demonstrated a close relationship between PRCV and transmissible gastroenteritis virus (TGEV) with the viruses being distinguished from each other only by the use of monoclonal antibodies (Laude et al., 1988; Garwes et al., 1988). Recently, Rasschaert et al. (1990) compared the genomic organization of each virus and showed that PRCV had various nucleotide deletions when compared to TGEV. No conclusion could be made concerning the genomic modification(s) controlling the phenotypic difference observed between the viruses, namely the impaired multiplication of PRCV in the digestive tract. Until now, great attention has

been given to clinical disorders induced by PRCV in the field (Jestin et al., 1987; Brown and Cartwright, 1986) and under experimental conditions (Vannier, 1990; Pensaert et al., 1986; O'Toole et al., 1989), but nothing is known about excretion and airborne transmission of the virus. In order to better understand that aspect of the epidemiology of PRCV, we studied excretion of the virus in pigs experimentally infected with the virus.

MATERIALS AND METHODS

Animals

Twenty one pigs from hysterectomy-derived sows were used. They were divided into two groups. One of twelve pigs was kept for experimental infection, and the other of nine pigs was used as control. The pigs were infected at 12 weeks old and an average weight of 41 kg. Each group was kept totally isolated from the other.

Virulent challenge

The strain isolated in France was used (Duret et al., 1988) as described by Vannier (1990). This strain was derived from nasal swab liquid, amplified by three passages in swine testis (ST) cell line, kindly provided by Dr. Brun from Rhône-Mérieux-Lyon. The cytopathic effect (CPE), induced by the virus, was neutralized by hyperimmune sera, specific for TGE virus and PRCV. The viral suspension was adjusted to an infectious titre of 10^6 $TCID_{50}$/ml. Each pig received 5 ml of the preparation by nasal (2 ml in each nostril) and oral routes (1 ml). The day of administration of virus was considered as day zero (D0).

Air sampling procedure

From day 1 to day 8 post-infection, air samples were obtained with a stainless-steel cyclone sampler, constructed according to the model described by Errington and Powell (1969). The air-flow rate of the apparatus was 300 l per minute. The airborne particles collected were eluted from the walls of the apparatus by addition of a collection medium with a peristaltic pump, adjusted to a flow-rate of 2 ml per minute. The cyclone sampler was placed about 1 m above floor level, between the two flat-decks each of which contained six pigs.

Four collection media were tested: Medium 1 contained phosphate buffered saline (pH 7.2) and buffer HEPES (20 mM); Medium 2 was medium 1 plus 10% foetal calf serum (FCS); Medium 3 was medium 1 plus 0.5% gelatine and Medium 4 was medium 3 plus 10% FCS.

Each medium was supplemented with penicillin 100 IU/ml, streptomycin 0.1 mg/ml and neomycin 50 IU/ml. Petri dishes, containing 10 ml of each collecting fluid, were placed daily in each isolation unit at about 20 cm above

floor level for 60 min. Parallelly, the cyclone sampler operated during 15 min for each medium. Ventilation of the loosebox was maintained during the sampling period.

Nasal swabs

Deep nasal swabs (about 10 cm) were collected from each pig, beginning on day 1 after infection and continuing for the next seven days. Each swab was weighed before and after collection in order to adjust the viral titres to a standard weight of 100 mg of mucus. Just after their collection, the cotton swabs were placed in 2 ml of minimum essential medium (MEM), supplemented with penicillin 100 IU/ml, streptomycin 0.1 mg/ml and with amphotericin B (125 μg/ml). Swabs were then stored at $-70°C$ until titration on ST cells in microtitre plates. Infectious viral titre was determined according the method of Kaerber (1931) and expressed in $TCID_{50}$/100 mg of nasal mucus.

Virus assay

Air samples were assayed on 24 h-old monolayers of ST cell line in 6-well plates (Falcon). Areas with CPE were counted and plaque forming units (PFUs) were calculated after 3 d at 37°C under 5% CO_2 and staining of the cell monolayer with methyl violet. Viral titres were expressed by the $\log_{10}$ value of PFU/m^3 of air. Samples were usually inoculated onto the cells a few hours after their collection. These results were compared with those obtained from deep nasal swabs.

Antibody assay

Blood samples were collected weekly from all pigs, starting one day before infection and continuing for the next three weeks. Porcine respiratory coronavirus and TGEV neutralizing antibodies were assayed using the technique described by Toma and Benet (1976) on ST cells. Antibodies titres were expressed by the inverse value of the highest serum dilution neutralizing 100 $TCID_{50}$ of the PRCV strain used for challenge.

Clinical observations, performance assessment

Clinical signs and, in particular, respiratory signs and rectal temperature were recorded daily. Each pig was weighed at D0 and D7, D14 and D21 after infection. Weight losses following infection and the duration of hyperthermia were the main criteria for evaluating the effects of the virus.

Necropsy

Three weeks after the experimental infection, the 12 pigs were slaughtered and necropsied.

RESULTS

Clinical signs

Only 9 of 12 pigs developed hyperthermia (rectal temperature $>40°C$) 24 hours after infection. Such temperatures were recorded only at D1 and D3. At that time, all animals were prostrated. Subsequently some animals, and not always the same ones, developed rectal temperatures reaching 40°C. Control animals developed no clinical signs of infection with PRCV.

Performance assessment

From D0 to D7, the difference of mean growth rates calculated between control and infected animals was statistically significant ($P<0.01$), with control pigs reaching a mean growth rate of 1095.24 g (SD=185.6) compared to infected pigs with only 726.19 g (SD=174.5). After D7, no difference between each group was noticed.

Serology

Antibody titres detected in pigs in each group are recorded in Table 1. All pigs seroconverted 2 weeks after infection, whereas control animals remained seronegative.

Air sampling

The quantities of airborne virus recovered from the isolated unit containing infected pigs are shown in Table 2. Virus was recovered for 6 days after infection with the highest recoveries between days 2 and 4.

The quantity of virus recovered each day varied with the collection medium used. Media 3 and 4 collected airborne viral particles for the longest period post-infection (until D6). A statistical comparison of both media demonstrated that medium 4 gave the best results, in relation to the duration of viral detection and quantity of virus recovered. A high correlation rate ($r=0.83$) was found between viral titres obtained from air samples collected with that medium and the amount of virus in nasal swabs (see tables 1 and

TABLE 1

Geometric mean of anti-PRCV antibody titres[1] detected weekly in pig sera in each group of pigs (infected and control)

Group of pigs	D0	D7	D14	D21
Infected	0	0	5.25 (1.21)	6.1 (0.9)
Control	0	0	0	0

[1]Antibody titres are expressed by the log2 value of the inverse serum dilution that neutralized 100 $TCID_{50}$ of virus.

3). Media 1 and 3, without proteins, were the least efficient in collecting significant viral titres during the sampling period.

Medium 2 was the most efficient considering the amount of virus collected, though virus was not detected before day 6. Table 4 records viral recoveries respectively obtained with each collection medium placed in settling plates. This collection method gave better results than the cyclone sampler i.e.: infectious virus could be detected from D1 to D6 with media 3 and 4, from D1 to D5 with medium 2 and only during the 3 days that followed infection in the case of medium 1.

TABLE 2

Viral recovery in air samples (cyclone) collected in the isolated unit containing pigs infected with PRCV during the 8 days test period, as a function of the collecting medium used.

Days p.i.	Cyclone sampler[1]			
	Medium 1	Medium 2	Medium 3	Medium 4
1	0.67	1.74	1.44	0.67
2	1.80	1.70	0.96	1.84
3	1.74	0.74	0.50	1.57
4	1.36	1.87	0.92	0.89
5	0.00	1.66	ND	1.26
6	0.00	0.00	0.96	1.14
7	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00

ND = Not done.
[1]Mean viral titres are expressed by the $\log_{10}$ value of PFU/m^3 of air.

TABLE 3

Geometric mean viral titres (expressed in $\log_{10}$ value of $TCID_{50}$/100 mg of mucus) obtained from nasal swabs during the test period.

Days p.i.	Nasal swabs titres
1	2.47 (1.60)
2	4.15 (0.81)
3	4.05 (0.39)
4	4.64 (0.34)
5	ND
6	3.05 (0.54)
7	2.02 (0.72)
8	0.24 (0.82)

ND = Not done.
( ) = Standard deviation.

TABLE 4

Mean viral titres calculated from petri dishes placed daily in each loosebox (viral titres are expressed by $\log_{10}$ PFU/h

Days p.i.	Medium 1	Medium 2	Medium 3	Medium 4
1	2.4	2.5	2.0	1.4
2	2.0	2.1	2.6	2.6
3	1.7	2.2	2.2	1.9
4	1.5	2.1	2.1	2.3
5	0.0	2.8	2.0	2.5
6	0.9	0.0	1.2	1.2
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0

Necropsy

Lungs of slaughtered animals were observed individually. No lesions were seen in control pigs or infected pigs.

DISCUSSION

Our results underline the difficulty of inducing severe disorders with PRCV in pigs under experimental conditions. Indeed, animals presented only slight clinical signs (hyperthermia, loss of weight) for a short period. This phenomenon was observed previously by authors such as Pensaert et al. (1986), O'Toole et al. (1989), Van Niewstadt et al. (1989) and Vannier (1990). The good health of the animals used in the experiments could explain the absence of respiratory disorders.

This is frequently observed with other viruses with respiratory tropism despite the absence of evident clinical signs, viral excretion occured during the test period.

The experimental procedure tested here has been used successfully by workers such as Donaldson et al. (1983) and Sellers and Parker (1969) respectively with Aujeszky's disease virus and with foot-and-mouth disease virus. Their findings showed differences in viral amounts excreted by infected pigs as detected in an all-glass cyclone sampler. Our results on the PRCV model are quite similar: airborne infectious virus could be recovered from air samples from D1 to D6 post-infection and in quantities closely related to those found in deep nasal cavity.

Environmental conditions, i.e. relative humidity (RH) and temperature (T), are of prime importance on virus stability, once exhaled into air (Cox, 1989). These parameters remained constant during the whole sampling period (RH=47%, T=20°C) in our experimental buildings and appeared to be suitable for maintaining the infectivity of PRCV.

Enveloped viruses, like coronaviruses, herpes viruses (Donaldson and Ferris; 1976, Schoenbaum et al., 1990) and myxoviruses (Schaffer et al., 1976), are known to be very sensitive to such environmental factors, mainly because of their outer phospholipid bilayer membranes. Phase changes occur when they are submitted to rehydration after desiccation, impaining or inactivating them. Indeed, lipid-containing viruses are well-known to be less stable at high RH.

The composition of the collection medium also seemed to be important for efficient collection of virus by cyclone sampling. Indeed, as demonstrated by Stolze and Kaaden (1989) for Sindbis and vesicular stomatitis virus, the addition of proteins or cryoprotective agents to sampling media improved the efficiency of collection of infectious virus. This was also demonstrated in the present study. Media 2 and 4, containing foetal calf serum, and gelatine + foetal calf serum respectively, produced the best viral recovery rates. A synergistic effect between each component could contribute to the stabilizing properties of these media during air sampling with the cyclone apparatus and with the petri-dishes.

Viral recoveries obtained with the settling-plates were relatively high, compared to those obtained with the cyclone device, suggesting that a major proportion of the airborne infectivity generated by the group of pigs was rapidly sedimenting, i.e., associated with large particles.

We did not attempt to measure the size of particles exhaled by the infected pigs. However data obtained by Robertson (1989) indicated that non-infected animals housed in the same isolation units generated large particles ($> 6\ \mu m$). This phenomenon is not surprising, considering the environmental atmosphere in which pigs were housed: food dust, faeces, skin squames and sneezing of animals, which is an important source of large aerosols.

The other important point of this study is the absence of correlation between viral excretion and clinical signs (mainly hyperthermia) i.e., the spread of PRCV among herd could be "silent". The relative vulnerability of the virus to environmental factors did not allow spreading in large amounts.

In conclusion, we can say that viral recovery directly from the air indicates airborne contamination better than viral titres calculated from nasal swabs. That latter collection technique reflects only a potential airborne viral quantity but not the real one.

The results we found with PRCV agree with Donaldson data with Aujeszky's Disease Virus (1983) but need some more investigation in field conditions.

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