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A SERO-EPIZOOTIOLOGICAL STUDY OF PORCINE RESPIRATORY CORONAVIRUS IN BELGIAN SWINE

M. Pensaert, E. Cox, K. van Deun, and P. Callebaut

SUMMARY
A porcine respiratory coronavirus (PRCV), antigenically closely related to transmissible gastroenteritis virus (TGEV), appeared in the European swine population in 1984. The present serological study was performed to obtain insight into the epizootiology of PRCV and of TGEV.

PRCV-induced neutralizing antibodies were found in 90.6 per cent of the 160 sera collected from sows at slaughter, demonstrating the enzootic appearance of PRCV in the Belgian swine population. A serological study of fattening swine on 33 farms revealed that 11 farms situated in an area with a high farm density (all farms within 4 km²) and 11 on 22 closed-breeding-fattening farms situated in areas with a low farm density (only one to four farms per 12 km²) were infected with PRCV throughout the year, whereas the other 11 closed breeding-fattening farms were temporarily free of PRCV. PRCV disappeared from the farms mainly in spring and summer. All the 11 farms became reinfected in autumn or winter, indicating that PRCV is regularly reintroduced in farms in the colder seasons. There was no correlation between the herd size and the temporary disappearance of PRCV from farms. It was observed on some farms that PRCV could infect pigs shortly after weaning in the presence of declining maternal antibodies, indicating that PRCV can persist on a farm by regularly infecting newly weaned pigs.

TGEV-specific antibodies were found in 7.6 per cent of the 160 sera from the slaughterhouse sows. TGEV-specific antibodies were also detected in sera from fattening swine of 5 of the above mentioned 33 farms. TGEV-outbreaks were not observed on these farms. During the last years, TGEV-outbreaks have rarely been diagnosed in Belgium. The present results suggest that TGEV infections still regularly occur but that they are not clinically manifest.

INTRODUCTION
A porcine respiratory coronavirus (PRCV), closely related antigenically to the enteropathogenic transmissible gastroenteritis virus (TGEV), emerged in the Belgian swine population in 1984 (15). TGEV multiplies in villous enterocytes in the small intestine causing villous atrophy and severe diarrhea. PRCV does not multiply in enterocytes in the intestinal tract of pigs of 4 to 5 weeks or older (5), but multiplies to a high titre in the respiratory tract (up to \(10^3\) TCID\(_{50}\) per g tissue) (6, 16). Infections with PRCV usually have a subclinical course. Nevertheless, in some studies respiratory disease in fattening pigs and sows has been attributed to infections with PRCV (8, 10, 11), and respiratory symptoms have been reproduced by experimental inoculation of fattening pigs (7, 16).

PRCV is transmitted by air and may remain infectious over many kilometres as evidenced by the sequential occurrence of PRCV infections on farms which are situated several kilometres apart and which apply strict sanitary measures (7,13). This has resulted in a rapid spread of PRCV since its appearance in 1984. From 1987 on, it has been extremely difficult to find Belgian swine farms which have not had a PRCV infection (13,14). On closed breeding-fattening farms, PRCV infections regularly occur in pigs younger than 3 months. The infections are observed especially during winter and early spring. It is not clear if the virus persists on these farms or is newly introduced with each wave of infection (15).

The increased prevalence of PRCV in the Belgian swine population has been accompanied by a decrease in the number of diagnosed TGE-outbreaks, suggesting that immunity against the widespread PRCV affects the behaviour of TGEV or that TGEV itself has been changed. However, it is still unclear whether the presence of PRCV results in a decreased prevalence of TGEV infections or only causes a milder course so that the infections are no longer clinically manifest.

Differential serological diagnosis between PRCV and TGEV infections is not possible with the classical sero-neutralization test, since infections with PRCV or TGEV induce antibodies which neutralized both viruses to the same titre. However, a competitive inhibition ELISA has been developed which permits serological differentiation between PRCV and TGEV infections. This assay is performed using a non-neutralizing monoclonal antibody directed against an epitope on the spike protein (S) of TGEV, which is absent on PRCV (4). Pigs infected with TGEV develop antibodies against this differential epitope, and these antibodies are detected with the competitive inhibition ELISA (3). The purpose of the present serological study was to obtain insight into the prevalence of PRCV and of TGEV. First, the incidence of PRCV and TGEV infections was determined by a serological survey of slaughterhouse sows in February 1990 and by a serological examination of fattening pigs on 33 farms. The latter study was performed between February 1989 and February 1990. Second, during the longitudinal serological study on the 33 farms it was examined whether the occurrence of PRCV infections is influenced by the location of the farm in a dense swine population, the herd size and the season. Third, it was investigated whether PRCV can persist on farms.

MATERIAL AND METHODS
Origin of the sera
1. Serological survey of slaughterhouse sows. The prevalence of PRCV and TGEV infections was determined by using sera from 160 sows, collected at slaughter in 1990. The sows originated from 81 different farms, namely 49 closed breeding-fattening farms, 23 breeding farms and 9 farms of which the type was unknown. These farms were located in the provinces of East-Flanders and Antwerp. Between one and five samples of sera were collected per farm.
2. Prevalence of PRCV and TGEV antibodies in fattening swine on 33 farms: influence of the swine population density, the herd size and the season

The prevalence of PRCV or TGEV infections on 33 farms was determined serologically. Eleven of the farms were located within an area of 4 km² accommodating a total of 18 farms and were designated farms in a densely populated area. Twenty-two farms were located in areas with not more than one to four farms per 12.5 km² and were designated widely dispersed farms. This distinction allowed us to determine the influence of the swine population density on the occurrence of PRCV or TGEV infections.

The 22 widely dispersed farms were all breeding-fattening farms. The number of sows and fattening swine on these farms are given in Table 2. The neighbouring farms, which are situated within a distance of 0 to 0.5, > 0.5 to 1 and > 1 to 2 km, respectively, of the widely dispersed farms, are also presented. Of the 11 farms located in a dense swine population, 5 were breeding farms with the number of sows varying between 45 and 105 and the number of 11- to 20-week-old pigs, between 20 to 150, 5 were closed breeding-fattening farms (in which pigs or sows from other farms were not introduced) with 30 to 300 sows and 190 to 1450 fattening swine, and 1 was an open breeding-fattening farm (11-week-old pigs from other farms were introduced) with 180 sows and 1500 fattening swine.

Four to eight sera were collected from 20- to 26-week-old swine on all 33 farms every 2 to 3 months between February 1989 and February 1990 (Table 1). The longitudinal serological survey allowed us to examine the influence of the season on the occurrence of an infection.

Table 1. A one-year serological study on the presence of PRCV-antibodies in 20- to 26-week-old fattening pigs on 22 breeding-fattening farms situated in areas with a low farm density.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of sows</th>
<th>Number of fattening pigs</th>
<th>Age of pigs at which pigs became infected with PRCV</th>
<th>Number of fattening pigs with PRCV antibodies at the time of infection</th>
<th>Number of fattening pigs with TGEV antibodies at the time of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>500</td>
<td>80</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>B</td>
<td>400</td>
<td>60</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>C</td>
<td>350</td>
<td>100</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>80</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>E</td>
<td>250</td>
<td>120</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>F</td>
<td>200</td>
<td>100</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>G</td>
<td>150</td>
<td>80</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>150</td>
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<tr>
<td>H</td>
<td>100</td>
<td>50</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>I</td>
<td>50</td>
<td>30</td>
<td>3, 5, 7, 9, 11 weeks</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

3. Age of pigs with maternal antibodies at the time of infection with PRCV

The approximate age at which pigs became infected with PRCV was determined serologically on three closed breeding-fattening farms (Farms A, B, C) and one breeding farm (Farm D). Sera were collected from different groups of eight to 10 pigs at the age of 3, 5, 7, 9 and 11 weeks. None of these pigs had TGEV-specific antibodies, as determined with the competitive inhibition ELISA (4). The four farms had between 100 and 200 sows, pigs on these farms were weaned between 3 and 6 weeks of age.

On farm A, an infection with PRCV occurred during the observation period, as diagnosed by virus isolation from tonsillar swabs of ten 5-week-old weaned pigs. In order to follow the course of the sero-neutralizing antibody titre (SN-titre) in these infected pigs, blood was collected at two-weekly intervals until the pigs were 13 weeks old.

Tonsillar swabs

Tonsillar swabs were collected from 228 pigs on farm A to determine the exact age of infection with PRCV. The swabs from groups of seven to ten pigs of the same age were pooled in 10 ml transport medium and stored at -70 °C until examined. The swabs were sampled from suckling pigs, namely five litters (39 pigs) of 3-week-old pigs, four litters (25 pigs) 4-week-old pigs and one litter (9 pigs) of 5-week-old pigs, and from weaned pigs, namely seven groups (69 pigs) of 4-week-old pigs, six groups (59 pigs) of 5-week-old pigs and three groups (27 pigs) of 7-week-old pigs. The 4-week-old weaned pigs remained in the breeding unit until they were 5 weeks old, whereas they were placed in a unit which contained 5- to 11-week-old pigs.

Demonstration of PRCV-antibodies and TGEV-specific 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-specific antibodies. Sera which did not contain the TGEV-differential antibodies, but which contained VN antibodies were considered to be positive for PRCV (PRCV-antibodies). Sera which contained VN and TGEV-differential antibodies were considered to be positive for TGEV (TGEV-specific antibodies).

1. Virus neutralization test

The virus neutralization (VN) test for the detection of common TGEV- and PRCV-antibodies was performed with SK6 cells, using the Purdue 114 strain of TGEV, as described previously (17).

2. Competitive inhibition ELISA

The competitive inhibition ELISA for the detection of differential serum antibodies directed against TGEV was performed as described previously (3).

Virus isolation

Virus isolation from tonsillar swabs was performed according to standard procedures. The swabs were shaken in transport medium for 1 hour at 4 °C. The transport medium consisted of phosphate-buffered saline supplemented with 10 per cent (V/V) foetal calf serum (GIBCO) and antibiotics. Subsequently, the suspension was centrifuged at 8000 g for 4 minutes at 4 °C. Then, the supernatant was inoculated onto 5-day-old swine testicle cell cultures (ST 6) (12). The monolayer was examined daily for cytopathic effects on 6 consecutive days. When a cytopathic effect was observed, the immunofluorescent test was performed using a hyperimmune serum against TGEV strain Miller conjugated with fluorescein isothiocyanate.

RESULTS

Prevalence of PRCV- and TGEV-specific antibodies in the sera of slaughterhouse sows

Virus-neutralizing antibodies were detected in 157 of 160 sera (98.2 per cent of the sera). The three sera without VN antibodies
were from three farms (3.7 per cent of the farms), namely two closed breeding-fattening farms and one breeding farm. One to three additional sera were collected of these three farms. All the additionally sampled sera contained PRCV-antibodies.

TGEV-specific antibodies were detected in 12 of 160 sera (7.5 per cent of the sera). The TGEV-specific sera were from 12 of 81 farms (14.8 per cent of the farms), namely 7 closed breeding-fattening farms, 4 breeding farms and 1 farm of which the type of production was unknown. From 8 of the 12 farms, one to four additional sera were collected which contained PRCV-antibodies. Additional sera were not sampled from the other 4 farms. PRCV-antibodies (VN antibodies and no TGEV differential antibodies) were detected in 145 of 160 sera (90.6 per cent of the sera). The PRCV-sera were from 77 of 81 farms (95 per cent of the farms).

Presence of PRCV- and TGEV-antibodies in fattening swine on farms: influence of the swine population density, the herd size and the season

TGEV-specific antibodies were detected in six sera sampled on five different farms, namely in June 1989 on two breeding-fattening farms (Farms V223 and V325) and in February 1991 on two breeding-fattening farms (Farms V282 and V310) and one breeding farm (E15). On the latter farm, two of eight sera contained TGEV-specific antibodies. The breeding-fattening farms were situated in the areas with a low farm density, and the breeding farm was in the area with the high farm density. The breeding farm had 77 sows and 20 female pigs of an age of 20 weeks. There were no signs of a TGE-outbreak on the farms during this study and there were no indications that a TGE-infection persisted on the farms.

There was no correlation between the presence of TGEV-specific antibodies and the number of swine on the farms, the distance to and the number of neighbouring farms.

PRCV-antibodies were demonstrated in all the sera sampled throughout the year on the 11 farms in the densely populated area, except for the two sera from farm E15 with TGEV-specific antibodies. The prevalence of PRCV-antibodies in sera from fatteners on the 22 widely dispersed farms is shown in Table 1. On 8 of these 22 farms, PRCV-antibodies were found in all the sampled sera, except for one sample from farm V325 with TGEV-specific antibodies. On the other 14 farms, PRCV-antibodies were absent in sera sampled on 21 occasions, namely during 1 of the 6 visits to nine farms, during 2 non-subsequent visits to one farm and during 2 or 3 subsequent visits to four farms. On 16 of these 21 visits, four of eight sampled pigs were seronegative. On five occasions, however, one or two of the four to eight sera contained SN-titres between 24 and 128, indicating that some pigs had been infected with PRCV.

In February 1989, three farms had pigs without PRCV-antibodies. In the following months the number of farms with seronegative pigs increased to reach a peak in June 1989. At that moment, seronegative pigs were detected on seven farms. Thereafter, the number of farms with seronegative pigs decreased to become zero in February 1990.

There was no correlation between the presence of seronegative pigs and the number of fattening pigs and/or sows on a farm, the number of neighbouring farms and the distance to these neighbouring farms (Table 1).

Age of pigs with maternal antibodies at the time of infection with PRCV

TGEV-specific antibodies were not detected in the sampled sera. The geometrical mean VN-titres (GMT) of the 3- to 11-week-old pigs on farms A, B, C and D are presented in Figure 1. On all four farms, the GMT was higher in the 3-week-old pigs (GMT of the four farms 96) than in the 7-week-old pigs (GMT of the four farms 32). On farm B, the titre further decreased in the 9- and 11-week-old pigs. Five pigs were seronegative at the age of 11 weeks and the GMT in the other five pigs was 6. On farms A, C and D no such decrease occurred. On farms A and C, the titres stopped decreasing between 7 and 9 weeks of age, indicating that a PRCV infection had occurred before the age of 7 weeks. On farm D, the increase occurred between 9 and 11 weeks of age, indicating that a PRCV infection had occurred before the age of 9 weeks.

On farm A, virus was isolated from tonsillar swabs from a group of ten weaned 5-week-old pigs. No virus was isolated from 3- to 5-week-old suckling pigs or from 3-, 4- and 7-week-old weaned pigs. The evolution of the GMT in the ten pigs from which virus was isolated followed the same pattern as the GMT detected in the sera from the 5- to 11-week-old pigs on this farm (Figure 1).

![Figure 1: Geometric mean PRCV-seroconversion-antibody titres in sera of groups of 8 to 10 pigs at the age of 3, 5, 7, 9 and 11 weeks, on three closed breeding-fattening farms (farms A, B and C) and one breeding farm (farm D).](image-url)
results of both studies demonstrate that PRCV is enzootic in the Belgian swine population. Similar observations have been made in other European countries (1, 8, 9, 16).

In the field study, the sampled fattening swine on a farm were either all seronegative, all seropositive or only one or two swine were seropositive with SN-titres equal to or higher than 24. It has been observed that pigs which become infected with PRCV in the presence of maternal antibodies have PRCV-SN-titres between 2 and 192 at the age of 5 months. In the absence of a PRCV infection, however, pigs with maternal PRCV-antibodies become seronegative by the age of 18 weeks (15). Therefore, 20- to 26-week-old fatteners were examined for the presence of PRCV and TGEV-specific antibodies. The observation that all sampled fatteners on a farm were seropositive indicates that a PRCV infection had occurred on this farm within a period of 4 to 5 months, whereas the presence of seronegative fatteners demonstrates that a PRCV infection had not occurred in the selected swine during a period of 4 to 5 months. On five farms, seronegative swine were present together with one or two swine with SN-titres equal to or higher than 24. Brown and Paton have made a similar observation (2). These high antibody titres in 5-month-old fatteners must have been induced by a PRCV infection. The fact that only one or two swine contained such high titres whereas the other two or six tested swine were seronegative suggests that older PRCV-immune pigs were present in a group of younger pigs that had not had a PRCV-infection or that a PRCV infection can remain limited to a group of animals on some farms. This should be further examined.

The sero-epizootiological study of 33 farms showed that PRCV can disappear temporarily from a herd. Two factors were observed to influence the occurrence of PRCV infections on a farm, namely the density of the swine population in the area in which the farm was situated and the period of the year. On 11 of the 22 widely dispersed farms the sampled pigs had not been infected for a period of at least 4 months, whereas PRCV was again demonstrated. The observation that all 11 herds became reinfected indicates that PRCV is regularly reintroduced to farms. The reinfection of the herds most likely occurs by the airborne transmission of virus from an infected neighbouring herd. Transmission has been reported to occur between farms situated several kilometres apart (13). An inverse relation has been observed between the risk of seroconversion and the square distance to a seropositive neighbouring farm. The risk increased with the size of the neighbouring herd, and became zero at a distance of 2000 metres (7). Virus transmission will thus occur more frequently between farms situated close to each other. This can explain why all the selected farms in the densely populated area were infected with PRCV throughout the year.

On 9 of the 11 widely dispersed farms with seronegative pigs, swine were seronegative during spring and/or summer. Almost all widely dispersed farms were infected with PRCV during autumn and winter. This observation shows that the season influenced the occurrence of PRCV infections, as has been reported previously (8, 15). Virus transmission between farms takes place more frequently during the colder than the warmer seasons.

A positive correlation between the risk of seroconversion in a herd and the herd size has been reported in a Danish investigation (7). This correlation was not seen in the present study, as farms with 280 as well as 1400 animals became temporarily free of infection. It should be noted that far fewer farms (33 farms) were examined in the present study than in the Danish report (410 farms), making it more difficult to demonstrate this correlation. Furthermore, a correlation between the herd size and seropositivity could have been masked by variations in the size and the seropositivity of neighbouring herds (7). Both factors were not determined in the present study.

Nearly all Belgian sows have PRCV-induced antibodies which are transmitted, via colostrum, to their offspring. In the absence of a PRCV infection, the maternal antibody titre declines with increasing age, with a mean half-life of 12.04 days (15), as was seen on farm B. On farms A, C and D, however, the decline was followed by a slight increase. This suggested that on these three farms the passive immunity was converted into an active immunity due to an infection with PRCV during the first weeks after weaning. This was proven on farm A by the isolation of PRCV from tonsillar swabs taken from 5-week-old pigs shortly after they had been introduced in an unit with feeder pigs. That virus was not isolated from younger or older pigs and that slightly higher PRCV-SN-titres were observed in 11- than in 9- and 7-week-old pigs at the moment of virus isolation indicates that PRCV persisted on farm A. Another argument in favour of the hypothesis that PRCV can persist on a farm is that 5-week-old pigs with maternal antibodies (SN-titres of 24 to 294) which are inoculated by aerosol will excrete PRCV for 8 to 13 days (Cox et al., unpublished observation). The weekly introduction of PRCV-susceptible animals to a unit, as occurred on farm A, can therefore result in the persistence of the infection in this unit.

The technical assistance of Ms. L. Sys., C. Van Marcke and M. Dorme is gratefully appreciated.

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REFERENCES


THE EFFECT OF ROTATIONAL GRAZING FOR PERIODS OF ONE OR TWO WEEKS ON THE BUILD-UP OF LUNGWORM AND GASTRO-INTESTINAL NEMATODE INFESTATIONS IN CALVES

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SUMMARY
An experiment was carried out with three groups of grazing calves and one house control group to study the effect of rotational grazing for periods of 1 and 2 weeks on the build-up of lungworm and gastro-intestinal nematode infestations respectively. The experiment demonstrated that rotational grazing for periods of 1 week on six plots prevented the build-up of heavy lungworm infections. A build-up of heavier lungworm infections was observed in a group that was rotationally grazed for periods of 2 weeks on three plots and a group which remained on one plot throughout the grazing season: there was no difference between these two groups. In all three situations, there was an adequate development of immunity against D. viviparum, as measured by worm recovery after challenge infection at the end of the experiment in comparison with worm recovery of the similarly challenged control group. Neither rotational grazing scheme protected the calves against gastro-intestinal helminthiasis, because trace calves, which grazed for 4 days only in August or October, acquired infections which would have resulted in severe illness or even death if necropsy had been postponed for a week.

INTRODUCTION
Infective larvae of the cattle lungworm Dictyocaulus viviparum are dispersed rapidly from faecal pats to pastures in the summer in North-West Europe, but their survival is short (4, 9). This could mean that the disease could be controlled effectively by rotational grazing of susceptible cattle for short periods on a sufficient number of plots. Such a grazing scheme was recommended in the former German Democratic Republic, where calves were kept on a pasture for 4-day periods and did not return to the same pasture within 40 days (6). A similar scheme for the prevention of lungworm disease by rotational grazing was described by Pouplaid (13). An earlier experiment showed that weekly rotational grazing of calves on six plots suppressed the build-up of lungworm infections (5). However, this experiment did not conclusively demonstrate that the calves acquired a sufficient degree of immunity. Moreover, this scheme did not prevent heavy infections with gastro-intestinal nematodes. We therefore repeated this experiment and tested another scheme in which calves were grazed for 2 weeks on three plots.

MATERIALS AND METHODS
In total 22 Friesian heifer calves were used, born in January 1990 and 6 born in May 1990.