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Sero-Epidemiological Survey of Porcine Respiratory Coronavirus (PRCV) Infection in Breeding Herds in Southeastern Spain

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With 4 tables

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Summary

In 1987 we conducted a sero-epidemiological survey in the Murcia Region (South-East Spain) to discover the prevalence and spread of PRCV-infection among breeding pigs and farms and determine the association between herd size and geographical zone with PRCV-infection. The Murcia Region was divided into four geographical zones and the farms classified by size in four categories. The random sample was statistically representative of both the breeding stock and farms in each geographical zone. We analysed 6,000 breeding pigs from 480 farms. The immunological techniques employed were indirect ELISA and blocking ELISA. The prevalence (P ± IC) of PRCV-seropositive breeding pigs and infected breeding farms was 14.53 ± 0.89 % and 21.87 ± 7.83 % respectively. On 55 % of the infected farms, the prevalence of seropositive breeding pigs was 60–100 %. PRCV-infection appears spread throughout the four geographical zones of the Murcia Region. However, a significant association ($p < 0.01$) was observed between geographical zone and the prevalence of PRCV-infection. A herd size of > 50 breeding pigs had a greater risk ($p < 0.01$) of PRCV-infection.

Introduction

In the last few years, a high incidence of TGE virus antibodies without clinical signs of enteric disease has been observed in the pig population of several European countries [Belgium (1); Great Britain (2); Denmark (3); France (4); Switzerland (5); Germany (6); The Netherlands (7); Austria (8); and Spain (9)]. Subsequently, a porcine respiratory coronavirus (PRCV) related to the TGE virus was isolated in Belgium (1), Great Britain (2), Denmark (10), France (11), Germany (6) and The Netherlands (7). Aerogenically transmitted porcine respiratory coronavirus has rapidly become widespread among the swine population and recently been identified as enzootic in several European countries (12).

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Although it was first considered as non-pathogenic (1), subsequent investigations have linked PRCV with field outbreaks of respiratory disease (4) and with pneumonia lesions following experimental infection (4, 7, 11, 13, 14).

The genome of the two viruses not only has a closely related organisation, but also presents a very low level (4 %) of nucleotide divergence (15). However, the PRCV genome presents two important distinctive features. The first was that the S gene lacks 672 nucleotides in the 5'region and encoded a truncated form of the S polypeptide (15); secondly, the first NS ORF down-stream of the S gene is predicted to be non-functional as a consequence of a double delection (15, 16, 17).

PRCV is a new variant of TGEV with an altered pathogenesis and epidemiology (1). Small changes in molecular structure between PRCV and TGEV are reflected in important changes in host cell tropism (18). PRCV multiplies mainly in the respiratory tract and infects only very few cells at the villus or crypt sites in the small intestinal mucosa and spreads from the ileum to the duodenum (13, 19, 20).

Antigenic studies have shown that this coronavirus is strongly related to TGE virus; the three non-structural proteins are quite similar. Nevertheless, differences have been identified by the use of appropriate monoclonal antibodies, and these mAbs show that several epitopes located in the C and D domains of gpS (Spike) of TGEV are absent or modified in the gpS of PRCV (21, 22, 23, 24).

Pigs infected with PRCV or TGEV cannot be distinguished by the conventional SN-test since both viruses show a complete cross-neutralisation activity. Nevertheless, differentiation is necessary for export to countries which require pigs to be free of TGEV-infection. PRCV has been found to be non-reactive towards several non-neutralising anti-TGEV MAbs directed against the S (Spike) protein. A differentiating blocking ELISA test has been set up using one or more of the non-neutralizing MAbs (22, 25, 26, 27).

In the present study we conducted a sero-epidemiological survey to discover the apparent prevalence of PRCV-infection in a random sample of breeding herds from South-East Spain, and determine whether geographical location and herd size were associated with an increased risk of PRCV-infection. PRCV antibodies were detected by indirect ELISA and subsequently by blocking ELISA.

Material and Methods

Epidemiological survey

The study was carried out in the geographical area of the Murcia Region, which we divided into four study zones: Cartagena, Guadalentin, Herta and Rest of the Region. The sample was made on both breeding farms and breeding pigs. The farms were divided into four categories according to the last census: family farms (1—9 breeding pigs), small (10—49), medium (50—99) and large (> 100).

The selection both of farms (based on size and geographical zone) and pigs to be analysed was made randomly.

Selection of herds

The Swine Brand Register (SBR) of the Murcia Region Board of Agriculture, Livestock and Fishery was used for selection of the sample. The SBR identifies herds by geographical area and includes data, up to 1987, on the number of breeding pigs in each herd. From the Register, all production herds (n = 6,281) were used as reference population.

A stratified analysis was conducted with two-way classifications according to geographical zone and herd size category (Table 1). For each combination of geographical zone and herd size category, the number of herds to be randomly sampled was always 30, in order to adapt to normal distribution. In total, 480 herds were studied (Table 1).

Selection of breeding pigs

The sero-epidemiological survey was conducted in 1987 to discover the apparent prevalence of TGEV-PRCV-infections, because these cannot be distinguished by the conventional serological test. We set the expected prevalence of infected breeding pigs at 30.9 %, which corresponds to TGEV-prevalence found by EGAN et al. (28) and HILL et al. (29) and which is similar to the mean prevalence

Table 1. Farms and breeding pigs analysed in the four herd sizes and geographical zones of Murcia Region

Herd size*		Geographical zone** I	II	III	IV	Total region
Family	Farms	30	30	30	30	120
	Pigs	150	150	150	150	600
Small	Farms	30	30	30	30	120
	Pigs	300	300	300	300	1,200
Medium	Farms	30	30	30	30	120
	Pigs	450	450	450	450	1,800
Large	Farms	30	30	30	30	120
	Pigs	600	600	600	600	2,400
Total	Farms	120	120	120	120	480
	Pigs	1,500	1,500	1,500	1,500	6,000

* Family (1—9 breeding pigs), small (10—49), medium (50—99) and large (100 or more). ** Geographical zones: I (Cartagena), II (Guadalentin Valley), III (Huerta of Murcia) y IV (Rest of Region).

reported by others (1, 30, 31, 32, 33). The frequency of PRCV-infections was already found to be high: 68 % (1) and 74 % (4).

In the herds with >30 breeding pigs, the number of pigs to be serologically analysed was calculated using the Binomial law formula (34).

$$n = \log (1 - C) / \log (1 - P),$$

where C = level of confidence,
and P = probability that a sampled pig was infected.

The estimate for each herd was 8 breeding pigs. To maximise the chance of detecting infected pigs in a herd, we increased the number of samples investigated to 10 in herds with 30—49 breeding pigs, 15 in the 50—99 category, and 20 in herds with >100 breeding pigs (Table 1).

The number of breeding pigs to be serologically analysed in the herds of <30 breeding pigs was calculated using the Hypergeometrical Distribution law formula (34).

$$1 - C = (Q \times N)! \, (N - X)! / N! \, (Q \times N - X)!$$

where C = level of confidence,
and P = probability that a sampled pig was infected,
Q = 1 − P,
N = number of breeding pigs in the herd,
X = size of sample.

The estimate for each herd was 10 breeding pigs. For herds with <10 breeding pigs, all the animals were tested.

In 1987, the breeding herds of the Murcia Region recorded a total of 188,774 breeding pigs. Of these, we analysed 6,000: from the family herds, 150 pigs per geographical zone; from the small herds, 300; from the medium herds, 450; and from the large herds, 600 (Table 1).

Serum samples

The blood sample were collected by the Veterinary Service of the Board of Agriculture and transported to the Regional Veterinary Laboratory. The blood was centrifugated at 1,500 × g and the serum conserved at −20 °C until analysis.

Immunological techniques

The diagnosis procedure was made in two steps: firstly, detection of coronavirus (TGEV and/or PRCV) antibodies by means of indirect ELISA (35); secondly, discrimination between TGEV or PRCV by means of blocking ELISA, using the monoclonal antibody 1DB12 as an indicator (25, 36). The monoclonal antibody 1DB12 was supplied by Dr. ENJUANES, Centro de Biología Molecular, Universidad Autónoma, Madrid, Spain (24).

Statistical analysis

Apparent herd prevalence was calculated as the proportion of randomly selected herds with one or more seropositive breeding pigs:

$$P = A/N \times 100,$$

where P = probability that a sampled herd was infected,
A = no of herds with > 1 seropositive breeding pigs,
N = number of randomly selected herds.

Apparent breeding pig prevalence was calculated as the proportion of seropositive breeding pigs from the randomly selected pigs.

$$P = A/N \times 100,$$

where P = probability that a sampled pig was infected,
A = no of seropositive breeding pigs,
N = number of randomly selected breeding pigs.

A 95 % confidence interval was estimated for the herd and breeding pig prevalence in the Murcia Region:

$$\text{Formula} = \pm Z\,(P \times Q)/N,$$

where Z = 1.96 for a 95 % level of confidence,
P = probability that a sampled herd or pig was infected,
Q = 1 − P,
N = number of randomly selected herds or breeding pigs.

The factors (herd size and geographical zone) presumably associated with the presence of PRCV-seropositive breeding pigs were tested at a 1 % level of significance by the chi-square test, for tables with four levels per factor.

Results

Prevalence of PRCV-infection in Murcia Region

PRCV-antibodies were detected in 872 (14.53 %) of the 6,000 breeding pigs studied. The seropositive breeding pigs came from 105 (21.87 %) of the 480 herds. On the basis of these 480 randomly selected herds, the apparent prevalence of PRCV-infection in the Murcia Region was 21.87 ± 7.38 % (95 % confidence interval). The apparent prevalence in breeding pigs was 14.53 ± 0.89 % (95 % confidence interval).

Table 2. Prevalence range of PRCV-seropositive breeding pigs in the infected herds

Range of prevalence	Zone I	Zone II	Zone III	Zone IV	Total
6–9 %	0	1	0	0	1
10–19 %	0	1	0	0	1
20–29 %	3	2	0	4	9
30–39 %	5	3	5	2	15
40–49 %	5	3	2	1	11
50–59 %	4	3	1	2	10
60–69 %	10	3	2	5	20
70–79 %	4	1	4	0	9
80–89 %	9	4	0	4	17
90–100 %	7	1	1	3	12
Total No	47	22	15	21	105
%	45	21	14	14	100

Table 3. Farms and breeding pigs censused, analysed and infected by PRCV in each geographical zone of the Murcia Region

	Geographical zone				
	Cartagena	Guadalentin	Huerta	Rest*	Total
Farms					
Censused	708	3,695	1,187	691	6,281
Analysed	120	120	120	120	480
Infected (no)	47	22	15	21	105
Infected (%)	39.16	18.33	12.50	17.50	21.87
C.I.**	8.73	6.92	5.91	6.79	7.38
Breeding pigs					
Censused	30,041	86,696	35,274	36,763	188,774
Analysed	1,500	1,500	1,500	1,500	6,000
Positive (no)	447	180	95	150	872
Positive (%)	29.80	12.00	6.33	10.00	14.53
C.I.**	2.31	1.64	1.23	1.51	0.89

* Cartagena, Guadalentin Valley, Huerta of Murcia, Rest of Region. ** C.I. confidence interval for security coefficient of 95 %.

Prevalence of seropositive breeding pigs on the PRCV-infected farms

The prevalence of PRCV seropositive breeding pigs in the infected herds ranged from 6.66 to 100 %. In a large proportion of infected herds (55 %), the prevalence of seropositive breeding pigs was high (60—100 %); 34 % of the infected herds showed a medium prevalence (30—59 %) and in only 10 % of the infected farms was the prevalence low (6—29 %) (Table 2).

Factors associated with PRCV-infection

Geographical zone. There was a significant association ($p < 0.01$) between geographical zone and the number of PRCV-infected breeding herds. The Cartagena zone

Table 4. Farms and breeding pigs censused, analysed and infected by PRCV in the four herd sizes of the Murcia Region

	Herd size*				
	Family	Small	Medium	Large	Total
Farms					
Censused	1,957	3,576	464	284	6,281
Analysed	120	120	120	120	480
Infected (no)	14	19	33	39	105
Infected (%)	11.66	15.83	27.50	32.50	21.87
C.I.**	5.74	6.53	7.98	8.38	7.38
Breeding pigs					
Censused	10,343	75,770	30,612	72,049	188,774
Analysed	600	1,200	1,800	2,400	6,000
Positive (no)	32	79	273	488	872
Positive (%)	5.33	6.58	15.16	20.33	14.53
C.I.**	1.79	1.40	1.65	1.61	0.89

* Family (1—9 breeding pigs); small (10—49); medium (50—99) and large (≥ 100). ** C.I. confidence interval for security coefficient of 95 %.

(39.16 ± 8.73 %) showed a more increased risk ($p < 0.01$) of infection than the Guadalentin Valley (18.33 ± 6.92 %), the Huerta of Murcia (12.50 ± 5.91 %) and the Rest of Region (17.50 ± 6.79 %) (Table 3).

Herd size. The association between the number of PRCV-infected breeding herds and herd size was also significant ($p < 0.01$). PRCV-seropositive breeding pigs were more likely to be detected in herds which housed more than 50 breeding pigs (50—99 category, 27.50 ± 7.89 %; > 100 category, 32.50 ± 8.38 %) than those in which there were < 50 (10—49 category, 15.83 ± 6.53 %; 1—9 category, 11.66 ± 5.74 %) (Table 4).

Discussion

The suspicion that the new TGE-related Porcine Respiratory Coronavirus (PRCV), which had spread almost uncontrollably throughout other European countries, had infected the swine population in South-East Spain, was scientifically proved in this study.

The absence or presence of blocking antibodies appears to be a reliable criterion for identifying antisera to PRCV or TGEV, respectively. The S protein epitope of TGEV defined by MAb 1DB12 is conserved by TGEV strains but in the field strains of PRCV is absent or modified (25).

In the mathematical formula (binomial law and hypergeometrical distribution law) used for determining the size of the sample, we considered an expected prevalence of 30.9 %, based on the known frequency of TGEV (28, 29). As the PRCV-prevalence of infection subsequently detected in the Murcia Region was lower (14.53 ± 0.89 %) than expected, we should have selected a bigger sample. The estimate for each herd was 8 breeding pigs; however, to maximise the chance of detecting infected pigs in a herd, we increased the number of samples investigated to 10 in herds with 30—49 breeding pigs, 15 in the 50—99 category, and 20 in herds with > 100 breeding pigs. This sample size was statistically significant (95 % confidence interval) for the prevalence of infection detected.

Of the random serum samples, 15.61 % were found PRCV- and/or TGEV-positive by indirect ELISA using the international reference strain Purdue (37, 38), and subsequently 14.53 % were found PRCV-positive when monoclonal blocking ELISA was applied. The prevalence of PRCV-infection in breeding pigs (14.53 ± 0.89 %) in the Murcia Region is similar to that reported by BEREITER et al. (5) in Switzerland (13 %) but lower than that of other surveys: 30.5 % in North-East Spain (36), 55 % in Austria (8), 68 % in Belgium (1), 73.70 % in France (4) and 87 % in Central Spain (9). The low prevalence of PRCV-infection in the breeding pigs (14.53 ± 0.89 %) and breeding herds (21.87 ± 7.38 %) of the Murcia Region may be explained by the recent appearance and spread of PRCV in the breeding herds of South-East Spain in 1987.

In the European swine population, after the spread of PRCV-infection, a lower or absent prevalence of TGEV-infections has been found in serological surveys. The prevalence of TGEV-infection, both on breeding farms (5.00 ± 1.94 %) and in breeding pigs (1.26 ± 0.28 %) in the Murcia Region (39) is similar to that (6 % infected farms and 1 % seropositive pigs) reported by BEREITER et al. (5) in Switzerland, but lower than that found in surveys conducted in other countries (1, 28, 29, 31, 32) before the appearance of PRCV-infection. When the blocking ELISA technique was applied in countries such as Austria, where surveys recorded high levels of seroreaction to TGEV, the positive reactions were observed to be due entirely to PRCV (8).

The present association in European countries between a high prevalence of PRCV and a low incidence of TGEV cannot in itself be taken as conclusive evidence of cross-protection, since TGE incidence has been known to fluctuate widely in the past (40). No evidence for cross-protection between immunity to PRCV and TGEV was found (40, 41, 42).

In countries where the PRCV has been isolated, the infection presents a wide geographical spread (43). PRCV is enzootic in the swine populations of Belgium (44), Great Britain (2), Netherlands (7) and Denmark (10). Likewise, PRCV-infection appears spread throughout the four geographical zones of the Murcia Region. However, we found

a correlation ($P < 0.01$) between the geographical zone and number of PRCV-infected farms. The farms in the Cartagena zone have a greater risk of infection.

HENNINGSEN et al. (45) found a pronounced positive association between the size of the herd as measured by the number of heat producing units (HPU) and the risk for porcine coronavirus seroconversion. Likewise, in our survey the size of the farm and the rate of contagiousness in the animals housed reveals an association ($p < 0.01$). The infection was more prevalent on farms with > 50 breeding pigs.

As this TGEV mutant only affects the respiratory tract of pigs under field conditions and, moreover, in mostly subclinical form, the dominant importance of this serological prevalence lies in the sectors of import and export certificates of a TGEV-negative status and in the way they are handled.

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