Seroprevalence of porcine respiratory coronavirus infection in Spanish breeding sows

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Abstract

The seroprevalence of porcine respiratory coronavirus (PRCV) among breeding sows was estimated in Castilla y León, the largest region in Spain, by a cross-sectional study. Serum samples (1247) were taken from sows from 58 different herds for this purpose throughout 1988 and were tested by a monoclonal antibody-capture ELISA (MACELISA), a test which detects antibodies to both transmissible gastroenteritis virus (TGEV) and PRCV. In order to discriminate positive sera to both coronaviruses, a blocking inhibition ELISA was used for all MACELISA-positive sera. By MACELISA, 31.4% of sera tested were positive and all of them were confirmed specifically as PRCV-positive by blocking inhibition ELISA, thus ruling out the presence of TGEV in the sampled area; 64% of the farms had at least one PRCV-seropositive sow, indicating that the infection was widespread Farms were classified into three categories, according to size and management practices. No significant differences were found in the prevalence rates among the three farm types; there was, however, a highly significant correlation between increasing farm size and increasing within-farm seropositivity.

Introduction

Porcine respiratory coronavirus (PRCV), a respiratory variant of transmissible gastroenteritis virus (TGEV), was first identified in Belgium in 1986 (Pensaert et al., 1986). PRCV is morphologically and antigenically similar to TGEV, but it has no tropism for the gut, replicating to high titers in the respiratory tissues of the pig and not inducing intestinal disorders. Instead, it produces mild or inapparent respiratory symptoms, and it affects the growth of fattening pigs (Bourgueil et al., 1992; Lanza et al., 1992). The humoral immune response elicited by both coronaviruses is indistinguishable (due to common neutralization epitopes) by the classical virus-neutralization test, but small antigenic differences that can be detected by monoclonal antibodies have allowed the development of differential serological tests (competitive

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inhibition blocking ELISA) for both viral infections (Callebaut et al., 1988a,b).

Since its first description in Belgium, PRCV infection has spread throughout Europe in a very short time, and it has been identified in the US (Wesley et al., 1990). Despite its limited pathogenicity, the epidemiological behavior and the factors associated with PRCV infection may serve as a model for other respiratory viruses, such as Aujeszky's or influenza viruses. The purpose of this study was to determine the seroprevalence of PRCV infection in the largest Spanish region (Castilla y León) and to learn about the factors related to PRCV seropositivity among swine herds.

Materials and methods

Sampling

The target population was the breeding sows of seven provinces of Castilla y León. Geographically, this region covers most of the NW–central part of Spain, and it is a plateau with an average altitude of 700 m above sea level. In April 1988, the breeding sow population in the sampled area was 327 972 animals (nearly 20% of the Spanish breeding sow census).

Farms were classified into three different categories depending both on the number of sows and the management practices, as follows:

(A) Family farms: this category included herds with 20–50 breeding sows, in which the piglets are usually sold to large fattening farms at weaning age. This type of farm is frequently found in certain parts of the sampled region and represents a secondary income source for many farmers. The production is not intensive and there is a great variability among farms in animal housing facilities, type of feed, management practices and hygienic conditions. Herds with less than 20 sows were not included in this study.
(B) Small intensive farms: herds containing between 51 and 100 breeding sows, intensively managed and either performing breeding only or a farrow-to-finish operation.
(C) Large intensive farms: farms with more than 100 breeding sows, intensively managed, performing a farrow-to-finish operation in which management and hygiene practices, type of feed and housing facilities are very similar among farms and generally satisfactory.

Fourteen family herds, 21 small intensive and 23 large intensive farms were selected and sampled. Within each herd, regardless of type, blood was taken from 20–22 sows. Each farm was sampled only once. Both herds and sows within herds were randomly selected from census data, using a random numbers generator.

A total of 1247 sows were sampled throughout 1988. Based on the formula $(n = Z^2 pq/L^2)$ of the approximate 95% confidence interval of the sample size for calculating the prevalence (Martin et al., 1987) the number of sows sampled allowed an absolute error (L) of 2.7% in determining the prevalence of infected animals at the 95% confidence level. As the expected prevalence (p) was unknown, 50% was chosen as the one giving the maximal sample size.

Blood was allowed to clot and the serum was separated by centrifugation, coded and kept at $-30°C$ until tested.

Serological tests

Serum samples were tested first by a monoclonal antibody-capture ELISA (MACELISA) (Lanza et al., 1993), a non-discriminating method which detects antibodies against both PRCV and TGEV. The sensitivity and specificity of this test, as compared with the standard virus neutralization test are 91% and 99%, respectively. In this test, samples were tested in duplicate wells, one containing viral antigen and the other negative control antigen, obtained from mock-infected cell cultures. ELISA values for this test were calculated as the optical densities in the viral antigen wells minus those in the control antigen wells. To assess the consistency of the test, a positive and a negative standard reference serum were tested in each plate. Positive sera were subsequently tested by a competitive inhibition blocking ELISA, as described by Callebaut et al. (1988b). This test, which uses a TGEV-specific monoclonal antibody as an indicator, specifically detects the humoral immune response produced by TGEV infection and is highly specific in detecting TGEV-seropositive pigs, its sensitivity (85%) being increased when using it on a herd basis.

Epidemiological/statistical analysis

The statistical analysis was performed taking the farm as the unit of concern. In order to determine if there were differences in the behavior of PRCV infection among the different farm types that could be explained by differences in its size or management practices, the following data analyses were performed.

Comparison of the prevalence of infected farms among the different herd categories. A farm was considered infected when at least one sampled sow was PRCV-seropositive. The number of PRCV-positive and -negative farms was compared among the three farm categories by the χ^2 test at $\alpha = 0.05$.

The occurrence of a positive correlation between herd size and within-farm seropositivity was analyzed with the Spearman's rank correlation. For this

purpose, three different ranks of within-farm seropositivity were established: low (1–32%), medium (33–66%) and high (67–100%).

MACELISA optical densities (ELISA values) were used as a measure of the amount of PRCV-specific antibodies in the serum samples. Then, mean ELISA values were compared among farm categories by Student's t-test at $\alpha = 0.05$. For most of the statistical analysis the version 5 EPI INFO computer package (Dean et al., 1990) was used.

Results

By MACELISA, 392 sows out of the 1247 samples (31.4%) were positive. These positive sera were subsequently tested by the differential blocking ELISA and all proved to be PRCV-specific. In 37 out of the 58 sampled herds (64%), at least one PRCV-seropositive sow was found.

The prevalence of infected farms was very similar among the different herd categories, ranging from 59% in large intensive to 71% in family herds. No overall significant differences were found between the different herd categories regarding the prevalence of infected farms (Table 1).

The mean percentage of infected sows per farm (within-farm seropositivity) increased in parallel with the farm size. This correlation was very significant by the Spearman's rank correlation test ($r_s = 0.99$, $P < 0.01$).

Table 1
Porcine respiratory coronavirus seropositivity in Spanish breeding sows

Farm type	No. sows tested	No. herds screened	Prevalence of infected farms (%)	Mean ± SD within-farm seropositivity (%)
Family	393	14	71	38.8 ± 33.9
Small intensive	391	21	62	60.7 ± 35.4
Large intensive	463	23	59	68.4 ± 31.5

Table 2
Student's t-test and significance values for the comparison of mean ELISA values between the three farm types

Farm type	Mean ELISA values ± SD	Compared with small intensive			Compared with large intensive		
		t	df	P	t	df	P
Family	542.8 ± 308.2	0.09	192	> 0.05	2.14	258	< 0.02
Small intensive	538.6 ± 262.0	–	–	–	2.99	324	< 0.01
Large intensive	451.7 ± 248.5	–	–	–	–	–	–

Mean ELISA values of sera belonging to each herd category are shown in Table 2; these values were similar for family and small intensive herds, but they were smaller in large intensive farms. Student's *t*-test showed that mean ELISA values in large intensive herds were significantly smaller than in the other two farm categories. No significant differences were found between family and small intensive herds.

Discussion

Neither TGEV nor PRCV has been previously recognized in the sampled area. In a limited sow sera survey performed with samples taken at the slaughterhouse in 1985, Rubio et al. (1987) did not detect TGEV neutralizing antibodies in any of the samples, thus ruling out the presence of both TGEV and PRCV in the area. Three years later, when our sampling was made, the situation seems to have changed completely, since 64% of the sampled farms had PRCV-seropositive sows. This confirms that PRCV spread rapidly among the swine population in Spain, similarly and concurrently to what happened in other European countries (Pensaert et al., 1986).

It is remarkable that TGEV-specific antibodies were not found in any tested animal. TGEV-specific antibodies have been found in sows of two different intensive pig-breeding areas of the Spanish Mediterranean coast (Murcia and Catalonia) (Cubero-Pablo et al., 1990) (M. Martin et al., personal communication, 1992). Many piglets are sent from Central Europe to these Spanish regions for fattening. Furthermore, at the time of our sampling, a TGE outbreak was diagnosed in the center of Spain, in a farm which had recently imported pigs from Belgium (Laviada et al., 1988). The reasons why TGE does not seem to have spread to Castilla y León remain obscure, but two facts could explain it: first, the entrance of TGEV infection by means of carrier pigs from other European countries seems unlikely, since piglets are rarely imported to Castilla y León from other European countries; second, the diffusion of TGEV from other parts of Spain is also unlikely, since pig trade between Castilla y León and other parts of Spain is mainly based on the export of piglets which are to be fattened in large intensive units located along the Mediterranean coast. On the other hand, the spread of PRCV seems to be much easier, since it is spread by airborne transmission (Wesley et al., 1990).

Significant differences were not found in prevalence rates across the different farm categories, suggesting that all types of farms are equally likely to be infected by the respiratory coronavirus, regardless of different management practices and probably due to the great aerogenic diffusion capacity of this virus. On the other hand, we found a very strong correlation between increasing farm size and increasing within-farm seropositivity, indicating that the transmission of this respiratory infection is easier within large animal popu-

lations. Henningsen et al. (1988) in Denmark, found that the probability of a pig being PRCV-seropositive increased while increasing the herd size.

Mean ELISA values were significantly lower in large intensive farms than in the rest, which might be explained by higher replacement rate of the sows in this type of farm, while smaller herds tend to keep the breeding animals for a longer time, thus increasing the probability of PRCV re-infections, which causes higher amounts of specific antibodies in serum and then higher ELISA values.

This study probably detected the beginning of a PRCV epidemic in the area, leading to a pandemic situation, as happened in the rest of Europe. Even though it is difficult to know what consequences this situation may have in the respiratory pathology of pigs, due to the low pathogenicity of this virus, it has to be taken into account as a part of the etiology of the swine respiratory complex. Also, despite the inconsistent cross-protection provided by PRCV on TGEV infection, this pandemic PRCV situation has occurred concurrently with a sharp decrease in the number of clinical TGE outbreaks in Europe.

Acknowledgments

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