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Seroprevalence of porcine epidemic diarrhea virus infection among different types of breeding swine farms in Spain

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Abstract

A cross-sectional study for determining the seroprevalence of porcine epidemic diarrhea virus (PEDV) infection among swine breeding farms in the Castilla y León region (Spain) was carried out. Breeding farms were stratified according to size and type of production (intensive or extensive). The number of farms to be sampled in each stratum was calculated from the latest census data available and the prevalence of seropositive farms obtained in other countries. Within each herd, the number of sows needed to detect the presence of the disease were sampled, according to within-herd seroprevalence data obtained by us in previous studies. A total of 5098 sow serum samples from 794 different farms were collected during 1992–1993 and tested for the presence of PEDV-specific antibodies, using a blocking ELISA with monoclonal antibodies; 55.9% of herds had at least one positive animal; 29.6% of sera were positive. Seropositive farms were detected throughout the sampled region, indicating that the infection was widespread. Higher farm-level prevalence rates were found in farms with more than 20 sows compared with small ones (≤ 20 sows) and in intensive than in extensive herds. On the other hand, within-farm seroprevalence and mean blocking percentage of positive sera decreased with increasing size of the farm.

Keywords: Pig; Porcine epidemic diarrhea virus; Spain

1. Introduction

Porcine epidemic diarrhea (PED) is a contagious gastroenteric disease of pigs with a clinical picture similar to that of transmissible gastroenteritis (TGE) and also caused by a

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coronavirus, porcine epidemic diarrhea virus (PEDV) (Witte et al., 1981; Pensaert and Callebaut, 1982). Two clinical forms of the disease have been described; PED Type I affects only pigs up to 4–5 weeks, while Type II outbreaks affect swine of all ages. Clinically, the disease is characterized by profuse watery diarrhea, high morbidity (close to 100%) and relatively low mortality, but still causing important economic losses to the pig producer (Pensaert, 1989).

Hofmann and Wyler (1988) described PEDV isolation for the first time in cell culture using Vero cells and trypsin-supplemented medium. Since then, several diagnostic methods have been described for the detection of both PEDV antigen and its antibodies (Hofmann and Wyler, 1990; Van Nieuwstadt and Zetstra, 1991; Knuchel et al., 1992).

In Spain, an outbreak of porcine gastroenteritis was described in 1985 in a province of Castilla y León (CyL) region and PEDV was identified as the etiological agent by electron microscopy and blocking ELISA (Jiménez et al., 1985). Since then, no further studies have been performed to determine the prevalence of PEDV infections in Spain, whereas prevalence of other swine coronaviruses like porcine respiratory coronavirus (PRCV) and transmissible gastroenteritis virus (TGEV) infections have been extensively studied (Rubio et al., 1987; Cubero et al., 1993; Lanza et al., 1993; Martín et al., 1994). The purpose of this study was to determine the seroprevalence of PEDV seropositivity among breeding swine farms from CyL, a region which concentrates nearly 25% of the Spanish breeding sow census, taking into account the different types of pig farms and management conditions.

2. Materials and methods

2.1. Sampling

The target of this survey was the farms housing breeding pigs of eight provinces of CyL. Breeding pigs were chosen because of their extended lifespan compared with fattening swine which increases the probability of contact with infectious agents and develop a serum antibody response (Müller et al., 1990).

CyL is the largest region in the European Community (EC), and includes most of the central and relatively high (700 m above sea level) plateau of Spain. It is divided into nine different provinces but all of them are geographically similar. In August 1991 the breeding sow population in the sampled area was 430 000 animals distributed over 39 522 farms, which represented nearly 25% of the Spanish and 2.4% of the EC breeding sow census.

Farms with a small number of animals are very important in this region as shown by the small mean number of sows per breeding farm (10.8). Therefore, to prevent sampling bias, farms were stratified into three different categories depending on the number of sows (a key factor determining both management practices and hygienic conditions) as follows:

(1) Small farms: farms with 20 or less breeding sows. There is great variability among this type of farm in the management practices and hygienic conditions, but they usually are deficient. These farms represent frequently a secondary income source for the farmers and so, conditions of housing facilities, type of feed, breed selection, etc. are rarely improved. Piglets are usually sold after weaning to large fattening farms. In the sampled area the number of small farms was 34 175.

(2) Medium size farms: herds containing between 21 and 100 breeding sows, generally with better management and hygienic conditions than the small ones. They perform either breeding only or a farrow-to-finish operation. The total number in the region was 4749.

(3) Large farms: this category includes 598 herds with more than 100 breeding sows, in which management practices and hygienic conditions are generally satisfactory. In this category, most of the farms finish their own pigs and use an all-in-all-out system.

In one of the sampled provinces (Salamanca), there is a particular extensive managed type of farms. These are herds with an average number of 70 sows of a particular breed of pigs (Ibérico). Extensive farms are mainly characterized by keeping the sows outdoors in the field during most of the production cycle, housing them only while nursing. During the survey we collected data about the extensive or intensive type of production in the sampled farms of Salamanca.

The number of herds to be sampled within each farm category for estimating the prevalence of infected farms was calculated using the EPISCOPE computer package (Frankena et al., 1990), taking data from the latest available census (August 1990) of swine breeding farms of CyL (data from the Consejería of Agriculture and Livestock of Castilla y León). We took 33% as the expected prevalence of PEDV seropositive breeding farms, as detected in a serosurvey carried out in Belgium (Pensaert, 1986) and we calculated that, with a 95% confidence level and an absolute error of less than or equal to 5%, a total number of 300 small farms, 300 medium farms and 175 large farms had to be sampled. The total sample size was proportionally divided according to the number of breeding farms within each of the sampled provinces.

The number of animals to be sampled within each herd was obtained from the formula of sample size for the detection of disease (Martin et al., 1987). We took 55% as the expected prevalence of seropositive animals within a herd, data obtained from two PED outbreaks diagnosed by us in this region, where we found a prevalence of seropositive pigs per herd of 53 and 60%, respectively, 3 months after the onset of the outbreak. The calculated number of serum samples to be taken per farm was three to four for small herds, between four and seven (maximum ten serum samples) for medium size farms and seven or greater for large farms (maximum 15 sera).

Both herds and sows within a herd were randomly selected from census data using a random number generator. Serum samples were provided by the Regional Animal Health Laboratory from samples submitted for the African swine fever eradication program. A total of 5052 serum samples from 803 different farms were collected throughout 1992–1993 with the following distribution: 983 sows from 309 small farms, 2317 sows from 319 medium size farms and 1752 sows from 175 large farms.

Blood was allowed to clot, centrifuged and the serum kept at −30°C until tested.

2.2. Serological test

PEDV-specific antibodies were detected in serum samples using a blocking-ELISA method, as described by Van Nieuwstadt and Zetstra (1991) with several modifications. Briefly, polystyrene microtiter plates were coated with monoclonal antibody (mAb) CVI-PEDV-66.31 and in a second step a mixture of gut-origin PEDV antigen and a 1:2.5 dilution of the test serum was added to each well. After washing, remaining free antigen (not blocked

by the test serum) was detected with a second mAb (CVI-PEDV 66.49) labelled with peroxidase. For each plate, four wells without serum were included as negative controls (blank), and two wells with hyperimmune anti-PEDV pig serum as positive controls. Then, the mean absorbance at 450 nm (A_{450}) of the four blank wells was calculated, and the blocking percentage for each test serum calculated from the formula:

$$\text{Blocking percentage} = 100\% - (A_{450}\ \text{serum} / \text{mean}\ A_{450}\ \text{blank}) \times 100\%$$

A sample was considered positive when blocking percentage was $\geqslant 50\%$.

Relative sensitivity and specificity of this test were 85.7% and 86.9%, respectively, when compared with a blocking ELISA described by Callebaut et al. (1982) using polyclonal antibodies (Carvajal et al., 1993).

2.3. Statistical analysis

The farm was taken as the unit of concern. A herd was considered positive when at least one of the sampled animals was positive and for each positive farm, within-farm seropositivity was calculated as the ratio between the number of positive sera and the total number of sampled animals in this farm. Also, mean blocking percentage of positive samples per farm was calculated.

The chi-square test at $\alpha = 0.05$ was used to detect any association between the prevalences of seropositive herds among the different farm size categories and between extensive and intensive production. The correlation between the prevalence of seropositive sows per herd (within-farm seropositivity) and the size of the farm was calculated using the Pearson product moment correlation for uncategorized variables.

The detection of significant differences between mean blocking percentages across the different farm categories was performed by ANOVA at $\alpha = 0.05$. For the statistical analysis the EPI INFO (v.5) computer package (Dean et al., 1990) and STATISTICA for Windows, v.4.0 (Statsoft, Inc.) were used.

3. Results

By blocking ELISA 1513 out of 5052 sera tested (29.9%) were seropositive. A total of 442 herds out of 803 sampled (55.9%) had PEDV seropositive sows (Table 1).

Comparing the prevalence of PEDV seropositivity across the different farm size categories, we found similar figures in large and medium size farms (65.1 and 67.8% positive herds, respectively). However, the prevalence of positive small farms (38.1%) was significantly lower ($\chi^2 = 16.8$ and 13.5, respectively, $P < 0.001$) (Table 1).

We found a highly significant negative correlation between farm size and within-farm seropositivity using the Pearson product moment correlation test ($r_s = -0.12$, $P < 0.001$), meaning that the number of positive samples per farm decreased significantly with increased size of the herd.

Since positive sera were not titrated, the blocking percentage was taken as a quantitative measure of the positivity of a particular sample. As shown in Table 1, mean blocking percentage of positive sera also tended to decrease while increasing the farm size. The

Table 1
Farms and within-farm seropositivity, and mean blocking percentages of positive sera to porcine epidemic diarrhea virus in breeding sows of Castilla y León (Spain) belonging to the three farm categories defined by the size

Farm category (no. of sows)	No. of herds	Positive herds		Within-farm seropositivity		Blocking percentage	
		Number	%	Mean	SD	Mean	SD
Small (<21)	309	118	38.1	56.9	27.1	71.1	10.4
Medium (21–100)	319	210	65.8	47.4	26.1	70.9	10.1
Large (>100)	175	114	65.1	46	27.4	66.5	8.3

A herd was considered positive when at least one of the sampled sows reached a blocking percentage ≥50% in the blocking-ELISA. Blocking percentage was determined for each positive farm as the mean of the blocking percentage of each of the positive sera. Within-farm seropositivity was calculated as the ratio between the number of positive sera and the total number of sampled animals in this farm.

Table 2
Farms and within-farm seropositivity, and mean blocking percentages of positive sera to porcine epidemic diarrhea virus in breeding sows of extensively and intensively managed farms in the Slamanca province (Spain)

Farm category	No. of herds	Positive herds		Within-farm seropositivity		Blocking percentage	
		Number	%	Mean	SD	Mean	SD
Intensive	75	24	32	47.8	25.5	70.8	10.1
Extensive	40	8	20	54.7	12.4	65.5	5.6

A herd was considered positive when at least one of the sampled sows reached a blocking percentage ≥50% in the blocking-ELISA. Blocking percentage was determined for each positive farm as the mean of the blocking percentage of each of the positive sera. Within-farm seropositivity was calculated as the ratio between the number of positive sera and the total number of sampled animals in this farm.

differences between small and medium size farms did not reach statistical significance, but the mean blocking percentage in large farms was significantly lower than in medium size ($F=8.56$, $P=0.003$) and small farms ($F=6.84$, $P=0.009$).

Comparing the prevalence of PEDV seropositivity between extensive and intensive farms in the province of Salamanca, the only where this type of production exists, we found significant differences (19.5% and 32% of positive herds, respectively) but no differences were found in either the mean blocking percentage or in within-herd seropositivity data between both types of production (Table 2).

4. Discussion

Although PEDV infection was described in the central part of Spain in 1985 (Jiménez et al., 1985), no studies have been performed since then to determine the importance of this infection among the Spanish swine population. During the last few years many acute diarrheal outbreaks affecting swine of all ages have been reported by practitioners in the

sampled area (J. Lamana, personal communication, 1990). In another serosurvey carried out in the same area in 1988, no TGEV-specific antibodies were found among breeding swine, thus ruling out TGE as the cause (Lanza et al., 1993). In the present study we have shown that PEDV infection is widespread among swine breeding population in the CyL region, which leads us to consider this virus as a potentially part of the etiology of diarrheal outbreaks.

The prevalence of PEDV-seropositive breeding sows found (29.6%) is similar to that reported by Debouck et al. (1982) in Belgium (32%), but much higher than the 5.6% prevalence found in Switzerland (Hofmann and Wyler, 1987). We also found a high prevalence of seropositive breeding farms (55.9%). There are no previous data about the prevalence in breeding farms in other countries, only from fattening or breeding and fattening farms from Belgium and Switzerland, with 33% and 28% of seropositive herds, respectively. The reasons why PEDV infection is so widespread among swine breeding farms in Spain remain unclear, but two facts could explain it. On the one hand, much of the replacement breeding stock is imported from other EC countries and also, Spain is a net piglet importer. All this traffic of animals would help in the dissemination of PEDV infection to other farms. On the other hand, the improvement of the screening methods, as compared with the ones used by other authors for epidemiological purposes, previous to the isolation of PEDV in cell culture, may explain a higher sensitivity and thus a higher number of positive animals detected.

Similarly to what has been described for TGE (Cubero et al., 1993), we found a higher prevalence of seropositive herds among the large and medium sized farms as compared with small ones. Taking into account that the entrance of PEDV in a farm is mainly by infected animals or by fecal-contaminated materials, large farms have a higher risk of infection because of the higher movement of animals, persons and vehicles.

We found a highly negative correlation between farm size and within-herd seroprevalence. The higher within-herd seroprevalence in small farms could be due to the easier spread of the infection among all the sows, since this type of farm normally houses the animals in only one barn throughout all the reproductive cycle and they do not use an all-in-all-out system. Also, in small herds, sows are usually not restrained, and this freedom of movement in the barn increases the probability of contact with fecal material from another sows. On the other hand, in large farms an average of 35% of the breeding sows are replaced annually and also this could explain the lower within-farm seroprevalence rates found.

Comparing mean blocking percentages between the different farm categories, we found significantly higher values in small and medium-sized farms than in large ones. PEDV infection was considered self-limiting, but recently, Pijpers et al. (1993) demonstrated that the virus can persist in a farm after an initial outbreak if there is a continuous input of susceptible animals. The lower antibody titer detected in large farms, as measured by the blocking percentage, together with the high prevalence of seropositive large farms could be a sign of the presence of endemic infections in this type of farm. Also, the lower replacement rate in small farms could increase the possibility for a sow to suffer a reinfection and develop a stronger antibody response.

Significant differences were also found in PEDV seropositivity among extensively and intensively managed farms in the province of Salamanca, with lower rates in the extensive

ones, as could be expected by the special breed and production system where there is a very limited interchange of animals between different farms.

This study confirms the importance of PEDV infection, which has been traditionally underestimated, in the etiology of enteric diseases of swine in Spain. We suggest that this virus should always be considered when diagnosing pig diarrhea.

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References

Callebaut, P., Debouck, P. and Pensaert, M., 1982. Enzyme-linked immunosorbent assay for the detection of the coronavirus-like agent and its antibodies in pigs with porcine epidemic diarrhea. Vet. Microbiol., 7: 295–306.

Carvajal, A., Diego, R., Lanza, I., Rubio, P. and Cármenes, P., 1993. Desarrollo de un ELISA de bloqueo para la detección de anticuerpos frente al virus de la diarrea epidémica porcina. Proc. 14th National Microbiol. Congress, September 1993, Zaragoza, Spain. Edited by the Scientific Committee of the Sociedad Española de Microbiología, Zaragoza, Spain, p. 179.

Cubero, M.J., León, L., Contreras, A., Astorga, R., Lanza, I. and Garcia, A., 1993. Transmissible gastroenteritis in pigs in south east Spain: prevalence and factors associated with infection. Vet. Rec., 132: 238–241.

Dean, A., Dean, J., Burton, E. and Dicker, R., 1990. EPI INFO, version 5: a word processing, database, and statistics program for epidemiology on micro-computers. USD, Incorporated, Stone Mountain, GA.

Debouck, P., Callebaut, P. and Pensaert, M., 1982. Prevalence of the porcine epidemic diarrhea (PED) virus in the pig population of different countries. Proc. 7th IPVS Congress, July 1982, Mexico. Edited by the Scientific Committee of the 7th IPVS Congress, p. 53.

Frankena, K., Noordhuizen, J., Willeberg, P., van Voorthuysen, P. and Goelema, J., 1990. EPISCOPE: computer programs in veterinary epidemiology. Vet. Rec., 126: 573–576.

Hofmann, M. and Wyler, R., 1987. Serologische Untersuchung über das Vorkommen der Epizootischen Virusdiarrhoe der Schwine (EVD) in der Schweiz. Schweiz. Arch. Tierheilkd., 129: 437–442.

Hofmann, M. and Wyler, R., 1988. Propagation of the virus of porcine epidemic diarrhea in cell culture. J. Clin. Microbiol., 26: 2235–2239.

Hofmann, M. and Wyler, R., 1990. Enzyme-linked immunosorbent assay for the detection of porcine epidemic diarrhea coronavirus antibodies in swine sera. Vet. Microbiol., 21: 263–273.

Jiménez, G., Castro, J.M., del Pozo, M., Correa, I., de la Torre, J. and Enjuanes, L., 1985. Identification of a coronavirus inducing porcine gastroenteritis in Spain. Proc. 9th IPVS Congress, July 1985, Barcelona, Spain. Edited by the Scientific Committee of the 9th IPVS Congress, p. 186.

Knuchel, M., Ackermann, M., Müller, H.K. and Kihm, U., 1992. An ELISA for the detection of antibodies against porcine epidemic diarrhoea virus (PEDV) based on the specific solubility of viral surface glycoprotein. Vet. Microbiol., 32: 117–134.

Lanza, I., Rubio, P., Fernández, M., Muñoz, M. and Cármenes, P., 1993. Seroprevalence of porcine respiratory coronavirus infection in Spanish breeding sows. Prev. Vet. Med., 17: 263–269.

Martín M., Casal, J., Lanza, I., Rubio, P. and Cármenes, P., 1994. Porcine respiratory coronavirus spread in Catalunya, Spain, a previously infection-free area. Prev. Vet. Med., 21: 65–74.

Martin, S.W., Meek, A.H. and Willeberg, P., 1987. Veterinary Epidemiology. Principles and Methods. Iowa State University Press, Ames, IA, 343 pp.

Müller, H.K., Ackermann, M., Bruckner, L., McCullough, K. and Kihm, U., 1990. Establishment and maintenance of a pig serumbank and its use in the surveillance of pig diseases in Switzerland. Proc. 11th IPVS Congress, July 1990, Lausanne, Switzerland. Edited by the Scientific Committee of the 11th IPVS Congress, p. 198.

Pensaert, M.B., 1986. Porcine epidemic diarrhea. In: A.D. Leman et al. (Editors), Diseases of Swine. Iowa State University Press, Ames, IA.

Pensaert, M.B., 1989. Porcine epidemic diarrhea. In: M.B. Pensaert (Editor), Virus Infections of Porcines. Elsevier, Amsterdam.

Pensaert, M. and Callebaut, P., 1982. Porcine epidemic diarrhea (PED) caused by coronavirus: present knowledge. Proc. 7th IPVS Congress, July 1982, Mexico. Edited by the Scientific Committee of the 7th IPVS Congress, p. 52.

Pijpers, A., van Nieuwstadt, A.P., Terpstra, C. and Verheijden, J.H.M., 1993. Porcine epidemic diarrhoea virus as a cause of persistent diarrhoea in a herd of breeding and finishing pigs. Vet. Rec., 132: 129–131.

Rubio, P., Álvarez, M. and Cármenes, P., 1987. Estudio epizootiológico de la gastroenteritis transmisible en Castilla y León. Proc. 8th Symp. of ANAPORC, October 1987, Barcelona, Spain, pp. 46–47.

Van Nieuwstadt, A.P. and Zetstra, T., 1991. Use of two enzyme-linked immunosorbent assays to monitor antibody responses in swine with experimentally induced infection with porcine epidemic diarrhea virus. Am. J. Vet. Res., 52: 1044–1050.

Witte, K.H., Prager, D., Ernst, H. and Neuhoff, H., 1981. Die Epizootische Virusdiarrhoe (EVD). Tieraerztl. Umsch., 36: 235–250.