ELSEVIER

Preventive Veterinary Medicine 21 (1994) 65–74

PREVENTIVE VETERINARY MEDICINE

Porcine respiratory coronavirus spread in Catalunya, Spain, a previously infection-free area

Margarita Martín[a,*], Jordi Casal[a], Ignacio Lanza[b], Pedro Rubio[b], Pedro Cármenes[b]

[a]*Patología Infecciosa i Epidemiología, Departament de Patología i Producció Animals, U.A.B., 08193-Bellaterra, Spain*

[b]*Enfermedades Infecciosas y Epizootiología, Departamento de Patología Animal (Sanidad Animal), Universidad de León, 24007-León, Spain*

(Accepted 2 February 1994)

Abstract

A retrospective study of sera from seven commercial pig herds was conducted from May 1985 to July 1989, showing that antibodies against Porcine Respiratory Coronavirus (PRCV) were first detected in Spain in September 1986. During 1991 a stratified sampling of breeding herds was conducted to establish prevalence to PRCV or Transmissible Gastroenteritis Virus (TGEV) infections in Catalunya (northeast Spain). An antibody-capture ELISA was used for PRCV- and/or TGEV-antibodies, while a competitive inhibition ELISA tested for TGEV-specific antibodies. This study revealed 91.1% positive sera and 96.7% positive farms to PRCV and/or TGEV with a gradual increase in prevalence during 1991, which was more noticeable in small herds. Of the total 569 herds tested, 65.2% were positive only to PRCV, 8.6% were positive to TGEV and 22.8% had mixed infections. The first appearance of antibodies coincided with large importation of feeder pigs from Belgium and Holland. This prevalence is much higher than previously reported in other regions of Spain.

1. Introduction

During the last decade, a new porcine coronavirus with tropism for the respiratory tract appeared in Europe. The appearance of a high prevalence of antibodies to Transmissible Gastroenteritis Virus (TGEV) without signs of diarrhea was first reported in Belgian herds in 1984 (Pensaert et al., 1986), followed shortly

* Corresponding author.

SSDI 0167-5877(94)00358-P

by most swine farms in Western European countries. The TGE-like virus isolate was designated TGE-like mutant (TLM-83) and named Porcine Respiratory Coronavirus (PRCV). Antibodies induced by this virus were identical to those of TGEV by a serum neutralization test, which resulted in interference with serological diagnosis of TGE.

A few years after the first isolation several European countries had 100% herd prevalence, while at the same time, exporting large numbers of pigs to other countries (Pensaert et al., 1987). At the present time, PRCV-infection is thought to occur throughout Europe. In North America a respiratory variant of TGEV, antigenically similar to European strains of PRCV, has been detected in pigs from both the USA (Wesley et al., 1990) and Canada (Jabrane et al., 1992).

The pathogenesis of this coronavirus has not been completely elucidated. Some authors believe that PRCV does not produce clinical signs but results in mild pneumonic lesions similar to those resulting from Influenza virus infection (O'Toole et al., 1989; Vannier, 1990; Cox et al., 1990; Lanza et al., 1992). In contrast, Duret et al. (1988) described fever and respiratory signs and Van Niewstadt and Pol (1989) reported lethal pneumonia in experimentally infected pigs.

Several epidemiological surveys have been made to determine the PRCV-seroprevalence in Spain (Yus et al., 1989; Cubero et al., 1990; Lanza et al., 1990), but the origin and evolution of PRCV-infection in herds was unknown. In order to establish when PRCV-infection appeared in Spain and its evolution during the following years, a retrospective survey was conducted. Furthermore, a sero-epidemiological study was made during 1991 to determine the TGEV- and PRCV-seroprevalence in the breeding farms of Catalunya.

2. Materials and methods

2.1. Retrospective survey

Catalunya is a Spanish region of 31 930 km^2 with approximately 4.75 million pigs, of which 537 000 are breeding animals in more than 10 000 herds. In order to determine the first appearance of PRCV-antibodies in this region, a retrospective survey using 544 sera was made from 8-week-old male pigs. The samples were obtained from seven herds located in Catalunya betweem May 1985 and July 1989, with the purpose of testing animals against several pathologies according to a breeding animals selection. Most of these sera (424 samples) were obtained from a performance-testing station between 1985 and 1986. This farm was considered to be a good sentinel for PRCV-infection since it periodically introduced pigs from other herds from around the entire region. Serum samples were only collected during the first week of quarantine previous to recording production in the test-station. Between 1987 and 1989 40 samples were collected from the same performance-testing station and also 80 samples were collected from six

other farms with the same objective, this completed the samples for the retrospective survey. Each pig was sampled only once.

2.2. Epidemiological survey

In order to know the prevalence of PRCV- or TGEV-infection, a stratified sampling of breeding herds was made. The three strata were: (1) Large (L), 100 or more sows; (2) Medium (M), between 20 and 99 sows; (3) Small (S), less than 20 sows.

Sample size was calculated taking into account the prevalence results from a preliminary survey with samples obtained from a slaughterhouse (92% for PRCV- and/or TGEV-infection, and 29% for TGEV, data not shown). The confidence interval (significance) was 95% and the degree of desirable precision was 4% for PRCV- and/or TGEV-infection and 6% for TGEV-infection for each sow-herd stratum. For a 95% confidence, the sample size was 210 for small and medium strata and 185 for large stratum.

Sera were obtained from official laboratories which test 30% of sows from all the farms of Catalunya every year to control and eradicate African Swine Fever. The farms for our study were selected in a simple random procedure using a computer-generated list. Finally, the real sample was 182 small farms, 223 medium farms and 164 large farms.

The sample size needed for detecting PRCV- and/or TGEV-infection in a herd was calculated, with a confidence level of 95% and a prevalence estimate of 33%, at eight sera for large and medium herds. For small farms all the sera obtained from routine official sampling were collected from 30% of all sows for each farm. A total of 3734 sera were tested.

To discriminate between PRCV- and TGEV-infection, two sera for each PRCV- and/or TGEV-positive farm were selected using a random table and tested by a differential ELISA. This sample is enough considering a 70% TGEV-antibody prevalence in the PRCV- and/or TGEV-positive farms with a 95% confidence level.

2.3. Statistical methods

Sample size (n) was calculated using the program EPI-INFO (Dean et al., 1990), according to the next formula:

$$N = z^2 \times p \times (1-p)/D^2$$

$$n = N \times P/(N+P)$$

where $z = 1.96$; P is population; p is estimated prevalence; D is precision level; N is sample for a infinite population.

The sample size to detect diseased animals was calculated with the same formula using a precision level to detect at least one positive serum, with a 95% confidence level.

The relationship between both animal and farm prevalences and the sow-herd strata and quarters of the year were calculated using the χ^2 analysis with Yates correction.

The confidence intervals for the prevalences were calculated according to the formula (Larson, 1988):

$$(x-p)^2=z^2\times p\times(1-p)/n$$

where x is prevalence; $z=1.96$; p is limit of probability; n is sample size.

2.4. Serological tests

An antibody-capture ELISA developed by Lanza et al. (1993) with a sensitivity of 91% and specificity of 99% relative to the serum-neutralization test was used in order to determine the prevalence of PRCV- and/or TGEV-infection. Briefly, ELISA plates (NUNC Maxisorp, Roskilde) were first coated with two monoclonal antibodies (6A.C3 and 1D.B12), directed towards different epitopes located in the peplomer (S) protein of TGEV (Jiménez et al., 1986). After overnight incubation at 37°C, the plates were washed and TGEV-antigen was added. Viral antigen was obtained by propagation of the attenuated Purdue strain on the swine testicle (ST) cell line and concentration at 100 000$\times g$ for 1 h at 4°C. Serum samples were added after inactivation at 56°C for 30 min and then the plates were incubated for 2 h at 37°C. After washing, horseradish-peroxidase-conjugated rabbit anti-swine IgG (Norden, Flow Laboratories, Madrid) was used. Incubation continued for 1 h at 37°C and finally chromogen substrate solution (0.005% H_2O_2 and 1 mg ml^{-1} 5-aminosalicylic acid) was added. The plates were read at a 450-nm wavelength using a Multiskan plus MKII reader (Titertek, Flow Laboratories, Madrid), with the cut-off value set at 0.100.

A competitive inhibition ELISA, performed according to Callebaut et al. (1988) using the monoclonal antibody 1D.B12 ascites fluid as the indicator antibody, was used to test sera for specific TGEV-antibodies. Positive samples by antibody-capture ELISA were tested by this competitive inhibition ELISA in order to differentiate between TGEV-positive sera and sera reacting only to PRCV. Briefly, ELISA plates (COSTAR High Binding, Badhoevedorp) were coated with an anti-TGEV-swine antiserum by overnight incubation at 37°C. After washing, TGEV-antigen was added and incubated for 3 h at 37°C. Then serum samples and appropriate controls were added in duplicate. Following overnight incubation at room temperature and without removing the contents of the wells, monoclonal antibody 1D.B12 was added and incubation was continued for a further 90 min at 37°C. The plates were washed and goat anti-mouse IgG1-horseradish-peroxidase-conjugate (Nordic, Tilburg) was used. The substrate solution was the same antibody-capture ELISA as described above. The minimal absorbance which would be obtained with TGEV-antibody negative sera was 45% of the mean results with an appropriate PRCV pool of sera taken as negative control serum. Samples with an absorbance below that value were scored positive for TGEV-antibodies.

3. Results

3.1. Retrospective survey

All samples taken before September 1986 were negative for TGEV- and PRCV-infection. From this date until December 1986, 64 positive sera were detected in the performance-testing station. None of these samples were positive to TGEV-antibodies by competitive inhibition ELISA. The distribution of negative and positive samples in relation to date of collection is shown in Fig. 1. The survey of 120 sera from the other six high-health herds showed seropositive animals continuously from 1987 to July 1989 (Fig. 2).

3.2. Prevalence of PRCV- and/or TGEV-infection

From 3734 tested sera obtained from breeding sow herds in 1991, 91.1% were positive by immunocapture ELISA. Prevalence in the total swine population was estimated to be between 90.1 and 92.0%. By grouping the samples into 3-month periods the results showed a gradual increase in the percentage of positive sera during the first three-quarters of 1991 ($\chi^2 = 21.57$, d.f. = 3, $P < 0.001$) (Table 1).

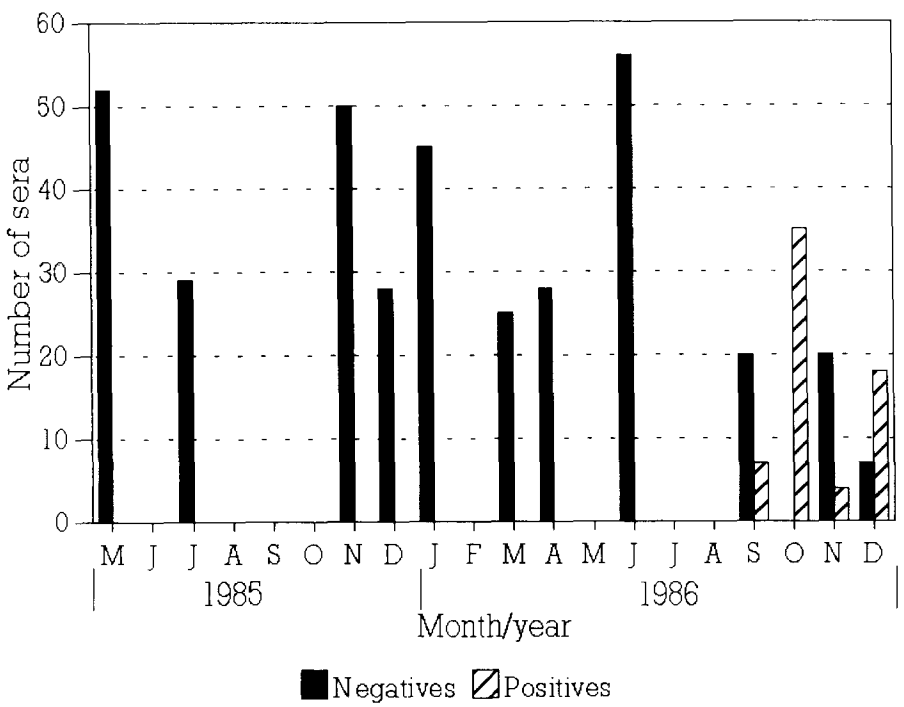

Fig. 1. Appearance of anti-Porcine Respiratory Coronavirus antibodies in a swine test-station in Catalunya, Spain.

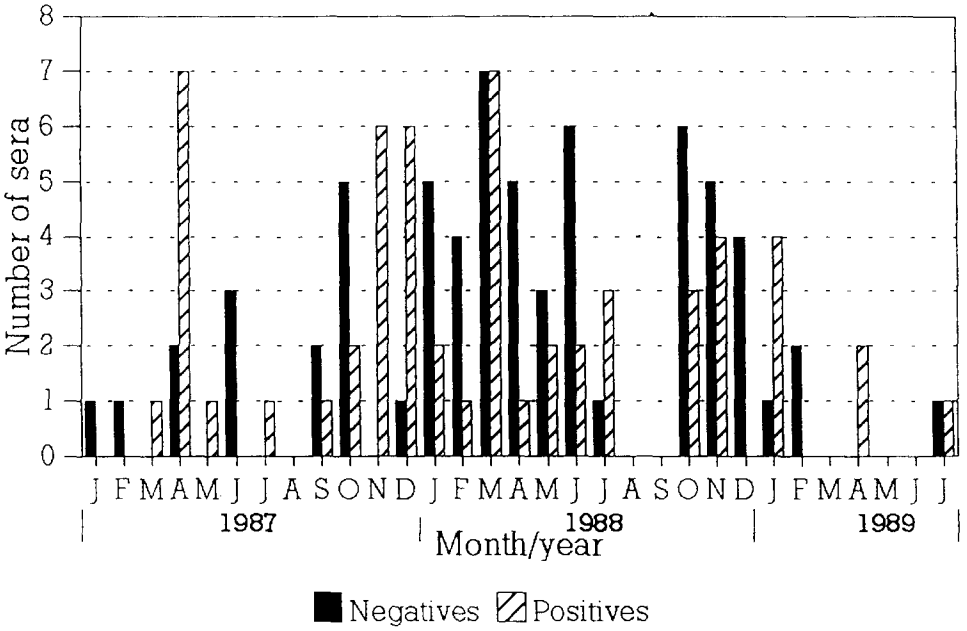

Fig. 2. Serological results against anti-Porcine Respiratory Coronavirus and/or Transmissible Gastroenteritis Virus by antibody-capture ELISA in several farms sampled retrospectively from Catalunya, Spain.

Table 1
Results of total sow sera tested against PRCV and/or TGEV by antibody-capture ELISA according to 1991 quarters in Catalunya, Spain

Quarter of 1991	Positive sera	Negative sera
1st	553 (87.6%)	78 (12.4%)
2nd	1297 (90%)	144 (10%)
3rd	801 (93.6%)	56 (6.5%)
4th	750 (93.2%)	55 (6.8%)
Total 1991	3401 (91.1%)	333 (8.9%)

($\chi^2 = 21.57$, d.f. $= 3$, $P < 0.001$).

A stratified analysis for every quarter of the year by grouping all the serum samples according to the farm size showed a lower prevalence in small herds with a remarkable increase through 1991 ($\chi^2 = 14.47$, d.f. $= 3$, $P < 0.01$) (Fig. 3).

Data from all 569 farms tested in 1991 by antibody-capture ELISA showed 550 with positive sera (96.7%). All farms with 100 or more sows had one or more positive sera by antibody-capture ELISA, whereas, 98.2% medium farms and 91.8% small herds were positive ($\chi^2 = 20.87$, d.f. $= 2$, $P < 0.001$). The smallest farms, however, showed an increase in prevalence from 85.7% in the first quarter of 1991, to 95.0% in the last quarter of the year, but never a 100% prevalence as

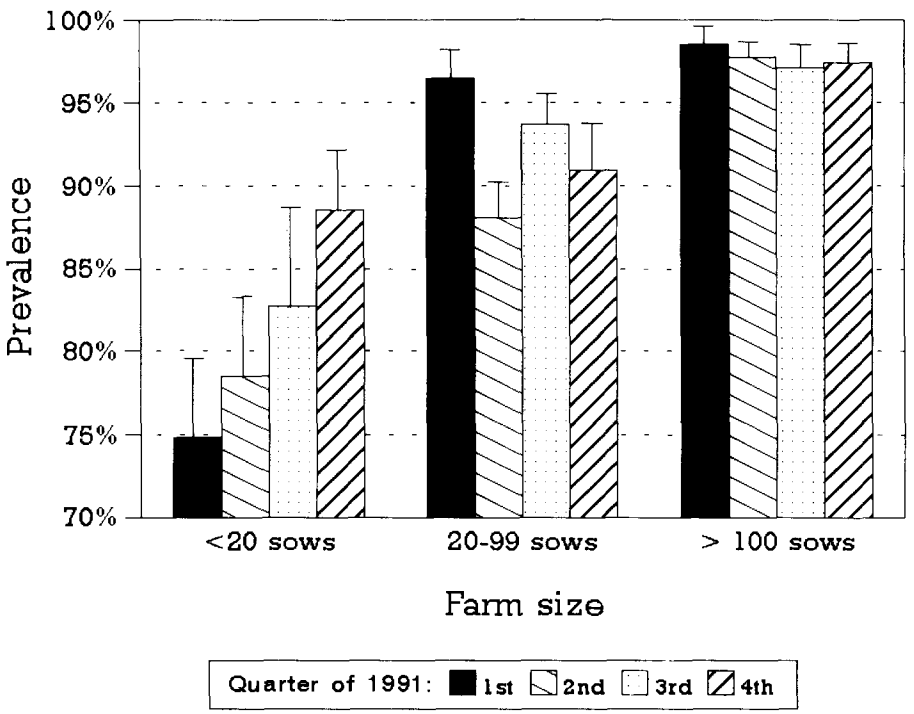

Fig. 3. Results of total sera against anti-Porcine Respiratory Coronavirus and/or Transmissible Gastroenteritis Virus, tested by antibody-capture ELISA according the size of the farms and the period of 1991 (error bars are 1 SE).

in the larger herds. The low number of the negative farms did not allow the calculation of the χ^2 value for the quarters considered.

3.3. Results by differential ELISA

In order to detect the presence of TGEV-infection, two sera from each of the 550 farms positive by antibody-capture ELISA were tested by differential ELISA. In 130 farms where the two samples gave different results all the sera (533 samples more) were then tested. Results for 1633 sera from these 550 farms were as follows: 403 (24.7%) sera were TGEV-positive; 1230 (75.3%) sera were TGEV-negative and PRCV-positive.

Finally, the 569 farms of the epidemiological survey were grouped according to the observed results. Detection of one TGEV-positive serum was considered as indicating infection in that farm from TGEV. If no positive sera were found, it was considered to be infected only by PRCV. The herds in which all sera were negative to both viruses were classified as negative farms (Table 2).

Table 2
Classification of pig breeding farms from Catalunya, Spain, according to serological results against Porcing Respiratory Coronavirus (PRCV) and Transmissible Gastroenteritis Virus (TGEV) with differential ELISA during 1991

Immune status of sow farms	No. of herds	Prevalence (%)	95% C.I.
Negative	19	3.3	1.9,4.7
Positive (PRCV and/or TGEV)	550	96.7	95.3,98.1
PRCV only	371	65.2	61.2,69.0
TGEV only	49	8.6	6.6,11.2
PRCV+TGEV	130	22.8	19.6,26.5

4. Discussion

PRCV-antibodies have been reported previously in other regions of Spain, although none of these studies were able to define the first appearance of infection in their area. Seropositive animals were detected in Murcia (southeast Spain) in 1987 (Cubero et al., 1990) in Castilla y León (northwest Spain) in 1988 (Lanza et al., 1990), and in the central area of Spain during 1989 (Yus et al., 1989). In the latter study, the authors did not use a differential technique, attributing antibodies detected by ELISA and serum neutralization tests to PRCV-infection.

The present study was performed in Catalunya (northeast Spain) in 1991. The high percentage of positive animals (91.1%), as well as positive farms (96.7%), indicate the extensive spread of PRCV- and/or TGEV-infection in different types of herds. Some of these sera were specifically positive to TGEV using the differential ELISA. They were also probably positive to PRCV, although this could not be assessed because a method to detect and differentiate specific antibodies to each of these two infections in the same sample was not available. With these data we can conclude that PRCV-prevalence in Catalunya was between 75% and 91% in 1991. This high prevalence is similar to those described in several European countries (Jestin et al., 1987; Bereiter et al., 1988), which reported high percentages of TGEV-antibodies by the serum neutralization test without an apparent increase in clinical cases of TGE.

These high prevalences, however, contrast with values reported in two recent epidemiological studies in Spain. Cubero et al. (1990) found only 14.53% of positive sera with 21.87% of herds affected in Murcia (southeast Spain), and Lanza et al. (1990) reported 30.5% of sows and 60% of farms being positive in Castilla y León (northwest Spain).

We found that 100% of large herds and 91% of small herds had animals with antibodies. This confirms the nearly complete spread of PRCV to all types of herds in Catalunya. Airborne transmission may have facilitated the dissemination of the virus between nearby herds, as the infection does not appear to always require carrier animals (Pensaert et al., 1987). We also found that the prevalence significantly decreased with decreased herd size. These results agree with data

reported in similar studies (Henningsen et al., 1988; Brown and Paton, 1991), which showed a faster spread in large industrial herds than in family farms. The different prevalence according to farm size could be explained by the fact that large farms often imported sows, increasing the risk of introducing carrier animals. In contrast, the smallest family herds usually obtained replacement sows from their own herd, and only exceptionally from large breeding farms nearby.

The present data together with published prevalence from other regions of Spain, suggest that: (1) PRCV-infection appeared initially in September 1986; (2) infection spread rapidly during the next few years; (3) in 1991 most of farms of Catalunya with more than 20 sows were infected, while infection in smaller herds increased during the year (from 75 to 88% of animals and from 86 to 95% of farms). It is probable that by 1992 the prevalences in these farms were similar to those in the larger farms.

The possible effects of PRCV-infection, either alone or associated with other pathogens, has not been elucidated (Lanza et al., 1993). The effect on fattening pigs where respiratory problems and the related delay in growth is a cause of important economic loss should be studied. In our study the only sign of PRCV-infection in sow units was the presence of antibodies which is of interest because of interference with TGE diagnostic tests.

Acknowledgements

We are grateful to Dr. Elizabeth Noyes and Dr. Carlos Pijoan for their help in the review of the english manuscript. The authors also thank the Departament d'Agricultura, Ramaderia i Pesca de la Generalitat de Catalunya which kindly provided sample sera for the epidemiological survey.

References

Bereiter, M., Hasler, J. and Keller, H., 1988. Transmissible Gastroenteritis (TGE) in der Schweiz: Antikorperpersistenz nach Infektion und seroepidemiologische Untersuchungen zur Bedeutung des TGE-Virus als Durchfallerreger. Schweiz. Arch. Tierh., 130: 237–248.

Brown, I. and Paton, D.J., 1991. Serological studies of transmissible gastroenteritis in Great Britain, using a competitive ELISA. Vet. Rec., 128: 500–503.

Callebaut, P., Correa, I., Pensaert, M.B., Jiménez, G. and Enjuanes, L., 1988. Antigenic differentiation between transmissible gastroenteritis virus of swine and a related porcine respiratory coronavirus. J. Gen. Virol., 69: 1725–1730.

Cox, E., Hooyberghs, J. and Pensaert, M.B., 1990. Sites of replication of a porcine respiratory coronavirus related to transmissible gastroenteritis virus. Res. Vet. Sci., 48: 165–169.

Cubero, M.J., León, L., Contreras, A. and Astorga, R., 1990. Epidemiological enquiry by serological survey (ELISA) of transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) in the region of Murcia (Spain). Proceedings of the 11th Congress IPVS, 1–5 July 1990, Lausanne. Edited by the Scientific Committee of the 11th IPVS Congress, Crissier-Lausanne, p. 264.

Dean, A.D., Dean, J.A., Burton A.H. and Dicker, R.C., 1990. Epi Info, Version 5: a word processing, database, and statistics program for epidemiology on micro-computers. USD Inc., Stone Mountain, GA.

Duret, C.H., Brun, A., Guilmoto, H. and Dauvergne, M., 1988. Isolement, identification et pouvoir pathogène chez le porc d'un coronavirus apparenté au virus de la gastro-entérite transmissible. Recl. Med. Vet., 164: 221–226.

Henningsen, D., Mousing, J. and Aalund, O., 1988. Porcint corona virus (PCV) i Danmark. En epidemiologisk tvaersmitsanalyse baseret pa screening-omrade sporgeskema data. Dansk Vet-tidssk., 71: 1168–1177.

Jabrane, A., Elazhary, Y., Talbot, B.G., Ethier, R., Dubuc, C. and Assaf, R., 1992. Porcine respiratory coronavirus in Quebec: Serological studies using a competitive inhibition enzyme-linked immunosorbent assay. Can. Vet. J., 33: 727–733.

Jestin, A., Leforban, Y., Vannier, P., Madec, F. and Gourreau, J.M., 1987. Un nouveau coronavirus porcin. Études séro-épidemiologiques rétrospectives dans les élevages de Bretagne. Recl. Med. Vet., 163: 567–571.

Jiménez, G., Correa, I., Melgosa, M.P., Bullido, M.J. and Enjuanes, L., 1986. Critical epitopes in transmissible gastroenteritis virus neutralization. J. Virol., 60: 131–139.

Lanza, I., Rubio, P., Muñoz, M., Callebaut, P. and Cármenes, P., 1990. Epidemiology of TGEV and PRCV infections in Spain. Proceedings of the 11th Congress IPVS, 1–5 July, Lausanne. Edited by the Scientific Committee of the 11th IPVS Congress, Crissier-Lausanne, p. 199.

Lanza, I., Brown, I.H. and Paton, D.J., 1992. Pathogenicity of concurrent infection in pigs with porcine respiratory coronavirus and swine influenza virus. Res. Vet. Sci., 53: 309–314.

Lanza, I., Rubio, P., Muñoz, M. and Cármenes, P., 1993. Comparison of a monoclonal antibody capture ELISA (MACELISA) to indirect ELISA and virus neutralization test for the serodiagnosis of transmissible gastroenteritis virus. J. Vet. Diagn. Invest., 5: 21–25.

Larson H.J., 1988. Introducción a la Teoría de Probabilidades e Inferencia Estadstica. Limusa, México D.F., México.

O'Toole, D., Brown, I., Bridges, A. and Cartwright, S.F., 1989. Pathogenicity of experimental infection with "pneumotropic" porcine coronavirus. Res. Vet. Sci., 47: 23–29.

Pensaert, M.B., Callebaut, P. and Vergote, J., 1986. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. Vet. Q., 8: 257–261.

Pensaert, M.B., Callebaut, P. and Hooyberghs, J., 1987. Transmissible Gastroenteritis Virus in Swine: Old and New. Jornadas Técnicas Expoaviga-87, Barcelona, pp. 40–45.

Vannier, P., 1990. Disorders induced by the experimental infection of pigs with the porcine respiratory coronavirus (PRCV). J. Vet. Med., Series B 37: 177–180.

Van Nieuwstadt, A.P. and Pol, J.M.A., 1989. Isolation of a TGE virus-related respiratory coronavirus causing fatal pneumonia in pigs. Vet. Rec., 124: 43–44.

Wesley, R.D., Woods, R.D., Hill, H.T. and Biwer, J.D., 1990. Evidence for a porcine respiratory coronavirus, antigenically similar to transmissible gastroenteritis virus, in the United States. J. Vet. Diagn. Invest., 2: 312–317.

Yus, E., Laviada, M.D., Moreno, L., Castro, J.M., Escribano, J.M. and Simarro, I., 1989. Prevalencia de anticuerpos frente a virus influenza y coronavirus respiratorio en cerdos de cebo en España. J. Vet. Med., Series B 36: 551–556.