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Risk factors associated with seropositivity to porcine respiratory coronavirus in Danish swine herds

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Abstract

Serological screening of swine herds in 1984 indicated that porcine respiratory coronavirus (PRCV) had been introduced into Denmark. To determine risk factors associated with the introduction of PRCV, a cross-sectional study of 408 Danish swine herds was carried out between May 1985 and June 1986.

The association between herd-PRCV serological status and possible risk factors, obtained from a field questionnaire, was assessed by unconditional maximum likelihood logistic regression. An increasing herd size, location in the Jutland peninsula (compared with location on the island of Funen) (OR = 7.9 in a multivariable logistic regression model not including interaction terms), the presence of a slurry system (i.e. pigs living on a slatted floor) (OR = 4.6) and purchase of pigs (OR = 1.7) were significantly (P < 0.05) associated with seropositivity. Two significant interactions, both involving herd size, were subsequently identified.

The PRCV serological status of neighbouring herds was found to be related, and closeness of a seropositive herd was associated with an increased risk of a herd being serologically positive.

The results of this study indicate that herd size may be an important determinant of airborne transmission of PRCV infection, and that herd size may modify the effect of other risk factors.

Keywords: PRCV; Risk factors; Airborne infections

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1. Introduction

Porcine respiratory coronavirus (PRCV), a virus closely related antigenically to the enteropathogenic transmissible gastroenteritis virus (TGEV), emerged in 1984 (Pensaert et al., 1986). The most striking differences between PRCV and TGEV are their site of replication and pathogenicity. PRCV principally multiplies in the respiratory tract, infecting epithelial cells and alveolar macrophages (Pensaert and Cox, 1989). Thus, PRCV has been detected in nasal swabs of experimentally infected pigs (Onno et al., 1989; Wesley et al., 1990) but not in the faeces, as is typical for TGEV infections. Microscopic examination of lungs reveals that PRCV causes a diffuse interstitial pneumonia (O'Toole et al., 1989; van Nieuwstadt and Pol, 1989; Cox et al., 1990). PRCV infection is generally subclinical or accompanied by mild respiratory disease, and villous atrophy is not observed in the intestines. TGEV, on the other hand, multiplies primarily in the epithelial cells covering the tips of the villi in the small intestine, causing diarrhoea.

PRCV infections can be serologically diagnosed with the classical seroneutralization test using TGEV as antigen (Witte, 1971). Titre values obtained after infection of pigs with TGEV or PRCV are similar, so differentiation is not possible if both viruses circulate in the herd. However, TGEV possesses antigenic sites in its S peplomer protein which are absent in PRCV (Callebaut et al., 1988; Garwes et al., 1988; Laude et al., 1988; Sanchez et al., 1990; Simkins et al., 1992). A differentiating competitive blocking ELISA has been developed using a non-neutralizing monoclonal antibody against this site of the S peplomer protein of TGEV (Garwes et al., 1988; Callebaut et al., 1989; van Nieuwstadt et al., 1989; Brown and Paton, 1991; van Nieuwstadt and Boonstra, 1992).

Since 1984, PRCV has spread rapidly and extensively through most countries in Europe (including Denmark), which has been explained in part by airborne transmission of the infection (Pensaert et al., 1993). The infection shows a seasonal pattern, infecting farms particularly during winter and early spring (Pensaert and Cox, 1989; Pensaert et al., 1993). However, PRCV may persist on a farm all the year round by regular infection of newly weaned pigs (Pensaert et al., 1993). At present, the virus is enzootic in the European swine population. For instance, PRCV-induced antibodies were detected in sera from 95% of the farms in Belgium (Pensaert et al., 1993).

This paper reports the results of a cross-sectional epidemiological study of 408 Danish swine herds that were all surveyed within 12 months after the initial introduction of PRCV into Denmark. The crude epidemiological results of the study were presented in a preliminary analysis by Henningssen et al. (1989). The objective of the present study was, by the use of multivariable techniques, to identify risk factors that may be associated with herd-level PRCV seropositivity.

2. Materials and methods

2.1. Study design and population

This cross-sectional study was carried out in the two regions of Denmark where PRCV was first diagnosed: the south of the Jutland peninsula and the island of Funen (postal
district numbers 5220 to 5853 and 6220 to 6470, respectively. To detect a difference in PRCV prevalence from 0.6 in exposed herds to 0.4 in non-exposed herds a sample of 100 exposed herds is needed (given a Type I error at 0.05 and a Type II error at 0.2, Martin et al., 1987). In order to detect differences of this magnitude for potential risk factors with high-to-moderate occurrence, a sample size of (approximately) 400 herds was set. The herds actually investigated \((n=408)\) were all volunteers selected by the 22 practising veterinarians in the two regions. The veterinarians were instructed to choose representative farms in their practice. The reference population of the two regions comprised 8295 herds, with an estimated total of 1 600 000 pigs (Landbrugsstatistik, 1986). All participating farms were visited once by their practising veterinarian between 24 May 1985 and 19 June 1986. During the visit, blood samples were collected and data on potential risk factors were obtained by questionnaire. Accordingly, both the producer and the interviewer were unaware of the PRCV serological status of the farm at the time of the interview.

2.2. Diagnosis

From all herds, a minimum of six blood samples from sows and 12 from fattening pigs were obtained (whenever possible) by the practising veterinarians. The veterinarians were instructed to select sows randomly, and to restrict the sampling of fattening pigs to a random sample of animals as close to slaughter weight as possible. Serum was examined in a virus-neutralization test at a dilution of 1/5 (mean of duplicate determinations) in a microtitre system against 100 TCID50 of FS 216/64 TGE-virus, using secondary porcine thyroid cells (Witte, 1971). As an indication of the test specificity, we found no positive reactions in 4396 serum samples from adult pigs tested prior to 1978. Tests were read after 3 days of incubation, and assigned as positive or negative. Because this test is not specific for PRCV it cannot normally be used to distinguish PRCV from TGEV. However, TGEV has never been diagnosed in Danish swine herds, either clinically, virologically or (using the aforementioned differentiating test) serologically, and therefore, seropositivity was defined as evidence of PRCV infection. A herd was considered infected when at least one pig tested positive for PRCV.

2.3. Potential risk factors

Risk indicators ascertained in the questionnaires were classified into four groups (Table 1): demographic variables concerning the herds, such as their size, the type of production, and the location and number of swine-producing farms within a radius of 3000 m; variables concerning the origin of feed and the presence or absence of a slurry system; management variables concerning purchase of animals, use of an external boar for breeding purposes (i.e. use of boars from a specialized boar station), presence of section(s) in the pig compartments, type of ventilation and presence of other animals on the farm; variables concerning the general herd health status, such as vaccination against respiratory diseases, presence of quarantine facilities for pigs introduced into the herd, presence of a room to change clothes and boots, contact with people, and herd health status with respect to SPF diseases. (In the Danish system, specific pathogen-free herds are free from \textit{Actinobacillus pleuropneumoniae} (several serotypes), \textit{Mycoplasma hyopneumoniae}, toxin-producing
Table 1
Description of variables used in the 1985–1986 cross-sectional study of porcine respiratory coronavirus (PRCV) in Denmark

Demographic variables
HPU = 0.17 × (pigs + fatteners) + 0.3 × (boars + gilts + sows)
Herd type (farrow-to-feeder herd, farrow-to-finish herd or fattening herd)
Geographical location (South of Jutland peninsula/island of Funen)
Neighbouring swine farms within a radius of 3000 m (0, 1, 2 or 3)

Feed and manure
Purchase of feed (yes/no)
Purchase of straw (yes/no)
Presence of a slurry system (yes/no)

Management
Purchase of pigs within the past 6 weeks (yes/no)
Use of an external boar (yes/no)
Presence of section(s) in the pig compartments (yes/no)
Type of ventilation system (forced/natural)
Presence of other livestock (yes/no)

Health status and biosecurity measures
Disease status (conventional herd/SPF or MS* herd)
Vaccination against respiratory diseasesb (yes/no)
Presence of quarantine facilities for pigs (yes/no)
Presence of room to change clothes and boots (yes/no)
Contact with people within the past 6 weeks (yes/no)

*Refer to Materials and Methods.
bActinobacillus pleuropneumoniae and/or Haemophilus parasuis.

Pasturella multocida, Serpulina hyodysenteriae, Sarcoptes scabei and Haematopinus suis. An MS herd is a herd with SPF status but infected with M. hyopneumoniae. All herds were free from Aujeszky’s disease (Pseudorabies), as documented by a national control program (Christensen et al., 1990).

A modified herd-size measure, the number of ‘heat-producing units’ (HPU), was used in the statistical analysis. It provides a method of comparing the sizes of different types of herds such as breeding, farrow-to-finish or fattening herds. One HPU (1 HPU = 1000 Watts at 20°C) is an estimate of the heat loss at various temperatures (and hence the ventilation requirement) for different age-classes of pigs. The formula used was

\[ 1 \text{HPU} = 0.17 \times (\text{pigs} + \text{fatteners}) + 0.3 \times (\text{boars} + \text{gilts} + \text{sows}) \]

where ‘pigs’ is the number of weaned pigs below 25 kg, and ‘fatteners’ is the number of fattening pigs above 25 kg (Strom, 1978).

2.4. Statistical analysis

A demographic herd-size comparison was made between the distributions of the reference population (Landbrugsstatistik, 1986) and the sampled herds. The distribution of herd sizes
was assumed to be multinomial when categorized into four groups (1–49, 50–199, 200–
499 and > 499 pigs). Using a $\chi^2$ test, the sample figures were compared with the regional,
population multinomial distribution, which was considered to be fixed. The $\chi^2$-test evaluates
whether the sample proportions may be considered identical in all four herd-size groups
(Haberman, 1979). A similar analysis was made of the distribution of within-herd sero-
positive reactions for the PRCV-positive study herds, with all results from within-herd
PRCV-positive tests in Denmark for the years 1985 and 1986 being used as a reference.
Here, the population and sample proportion of seropositive animals were divided into ten
equal-sized percentile groups.

The epidemiologic analysis was carried out with the herd as the unit, and examined the
relationship between herd characteristics and PRCV herd status. The SAS program (Statis-
tical Analysis Systems Institute Inc., 1990) was used for data management. The independent
variables (Table 1) were categorial or continuous. To stabilize the variance of the variable
HPU it was transformed to a natural logarithm.

The procedure PROC LOGISTIC of the SAS program (SAS Institute Inc., 1989) was
used to fit a multiple logistic regression model using the unconditional maximum-likelihood
method. Initially, a model was fitted with the herd serological status as the dependent variable
and all of the 17 variables in Table 1 as (potential) independent variables. All were included
as main effects, with no interaction terms. Because of some missing values, only 316 of the
408 herds were used in the statistical modelling. No differences were found between the
model dataset (316 observations) and the full dataset with respect to the marginal distri-
butions of the dependent and the 17 independent variables (data not shown). In a stepwise
approach, one variable at a time was added (forward selection) or omitted (backward
selection) from the model with all 17 variables, employing a likelihood ratio test at each
step. For each variable, the significance level for entry into or removal from the model was
0.15. After this procedure, four variables remained in the model.

All possible interaction terms between the four previously selected variables were sub-
sequently tested using a stepwise selection process as described above. After this screening,
two interactions remained in the model.

Because of the choice of $\ln$(HPU) as the herdsise measure, more weight is given to
boars, gilts and sows than to pigs and fatteners. Therefore an alternative modelling strategy
was applied, using the natural logarithm of the total number of pigs (the sum of animals
below and above 25 kg, boars, gilts and sows) as the herd size measure.

A dataset was prepared consisting of 206 herds that had a neighbouring swine herd
(within a radius of 3000 m) identified from among the 408 herds in the full dataset. From
this dataset, a sub-set was created consisting of 125 herds that had a PRCV seropositive
neighbouring herd. The risk of a herd being seropositive was analyzed by logistic regression,
with the independent variables: $\ln$(HPU of outcome herd); location of herd (Jutland pen-
insula or island of Funen); purchase of pigs in the past 6 weeks; $\ln$(HPU of neighbouring,
seropositive herd); distance (in units of 100 m) to neighbouring, seropositive herd. The
same modelling strategy as previously described was used in this analysis. However, owing
to sparseness of data, only main effects were included.

The significance of the coefficients in the final models was estimated by Wald's $\chi^2$-test.
Coefficients were exponentiated to obtain point estimates of adjusted odds ratios for cate-
3. Results

3.1. Crude analysis

The herd sample proportion relative to herd-size (proportion of sampled herds in a herd-size group divided by proportion of population in the same group) is shown in Fig. 1. The sample proportions differed significantly for the Jutland area ($\chi^2 = 30.3, 3$ d.f., $P < 0.001$), but not for Funen ($\chi^2 = 0.76, 3$ d.f., $P = 0.85$).

The mean number of blood samples per herd was 17.2. 61.5% of the 408 herds tested positive, with a mean prevalence of positive pigs of 90.0%. 78.5% of the positive herds had 100% seropositive pigs. The distribution of the within-herd seropositive reactions for the positive study herds is shown in Fig. 2, with all results from within-herd PRCV-positive tests in Denmark for the years 1985 and 1986 as a reference. The two distributions did not
differ significantly ($\chi^2 = 14.4$, 9 d.f., $P = 0.11$). PRCV seroprevalences by risk factors are indicated in Table 2.

3.2. Logistic regression model

Four of the variables were positively associated with PRCV seropositivity in the initial logistic model: $\ln(\text{HPU})$ ($\beta = 0.52$, $P < 0.001$); location in the Jutland peninsula ($\beta = 2.07$, $P < 0.001$); presence of a slurry system ($\beta = 1.52$, $P = 0.054$); purchase of pigs within the past 6 weeks ($\beta = 0.51$, $P = 0.109$).

However, two significant interactions were also identified: between $\ln(\text{HPU})$ and geographical location, and between $\ln(\text{HPU})$ and slurry system (Table 3). Because of this, the estimation of the odds ratio for HPU using this model is complicated (Table 4). An increase in HPU from the first to the third quartile increases the odds differently with respect to the location of the herd and to the presence or absence of a slurry system.

Reanalysis of the data using $\ln(\text{number of pigs})$ instead of $\ln(\text{HPU})$ generally produced only minor changes in coefficients, and the same four main effects and two interactions as before were significant (data not shown).

3.3. Analysis of neighbouring swine herds

In the dataset including 206 herds with any identified neighbour (among the 408 herds examined), a marked positive association between neighbouring herd seropositivity and the herd's serological status could be detected ($\text{OR} = 10.2$, $P < 0.001$). The results of the analysis of the 125 herds with a known seropositive neighbouring herd are shown in Table 5. Only $\ln(\text{HPU})$ of the outcome herd and the distance to the neighbouring seropositive herd were significant in the logistic regression model.
Table 2  
Seroprevalence of PRCV in 408 Danish swine herds (1985–1986)

| Variable                                           | No. herds | % PRCV-positive |
|----------------------------------------------------|-----------|-----------------|
| **Demographic**                                    |           |                 |
| ln(HPU) (heat-producing units)                     |           |                 |
| 50% smaller herds ( < 3.31)                        | 202       | 43              |
| 50% larger herds ( ≥ 3.31)                         | 203       | 81              |
| **Herd type**                                      |           |                 |
| Farrow-to-feeder                                   | 120       | 61              |
| Farrow-to-finish                                   | 160       | 58              |
| Fattening                                          | 105       | 68              |
| **Geographical location**                          |           |                 |
| Island of Funen                                    | 213       | 42              |
| Jutland peninsula                                  | 195       | 83              |
| **Neighbouring swine farms within a radius of 3000 m** |   |                 |
| No herds                                           | 79        | 61              |
| 1 herd                                             | 156       | 70              |
| 2 herds                                            | 102       | 61              |
| 3 herds                                            | 71        | 45              |
| **Feed and manure**                                |           |                 |
| Purchase of feed                                   |           |                 |
| No                                                  | 42        | 57              |
| Yes                                                 | 359       | 63              |
| Purchase of straw                                  |           |                 |
| No                                                  | 370       | 62              |
| Yes                                                 | 31        | 64              |
| Slurry system (slatted floor)                       |           |                 |
| No                                                  | 326       | 56              |
| Yes                                                 | 39        | 90              |
| **Management**                                     |           |                 |
| Purchase of pigs within the past 6 weeks           |           |                 |
| No                                                  | 258       | 54              |
| Yes                                                 | 150       | 74              |
| Use of an external boar                             |           |                 |
| No                                                  | 374       | 63              |
| Yes                                                 | 34        | 44              |
| Presence of section(s)                              |           |                 |
| No                                                  | 278       | 55              |
| Yes                                                 | 101       | 85              |
| Type of ventilation system                          |           |                 |
| Natural ventilation                                | 118       | 39              |
| Forced ventilation                                 | 290       | 71              |
Table 2 (continued)

| Variable                                      | No. herds | % PRCV-positive |
|-----------------------------------------------|-----------|-----------------|
| Other livestock than pigs                    |           |                 |
| No                                            | 141       | 69              |
| Yes                                           | 258       | 59              |
| Health status and biosecurity measures         |           |                 |
| Disease status                                |           |                 |
| SPF/MS herd                                   | 52        | 81              |
| Conventional herd                             | 356       | 59              |
| Vaccination against respiratory disease       |           |                 |
| Yes                                           | 103       | 80              |
| No                                            | 305       | 55              |
| Quarantine facilities                         |           |                 |
| No                                            | 359       | 62              |
| Yes                                           | 24        | 83              |
| Room for people to change clothes             |           |                 |
| No                                            | 308       | 58              |
| Yes                                           | 74        | 85              |
| Contact with people within the past 6 weeks   |           |                 |
| No                                            | 215       | 61              |
| Yes                                           | 193       | 62              |

4. Discussion

The study sample included 408 herds, or 16% of the herds in the whole area. The risk of possible misclassifications and the validity of the study group as compared with the population should both be considered. With respect to the serological classification (infected/non-infected), we found a within-herd prevalence of positive reactors at 0.9, leaving high confidence in a negative herd-level serological diagnosis. With respect to external validity, the PRCV-positive study herd serological profile was similar to all PRCV-positive herds (Fig. 2). However, differences were found in herd size (Fig. 1), so selection of large herds in the Jutland peninsula emphasizes the effect of large herds in the study.

Herds located in the Jutland peninsula were more likely to be seropositive than herds located on the island of Funen. An 'epidemiological corridor' probably exists between the north of Germany and the south of the Jutland peninsula. Such long-distance airborne virus transmission has also been suggested for Aujeszky's disease virus (Christensen et al., 1990). The type of floor where pigs were raised influenced the odds of the herd being seropositive. This result cannot be related to the introduction of PRCV into the herd. However, slatted floors may be considered to be a stress factor which might decrease pigs resistance to respiratory diseases. Similar results were found in studies of pneumonia (Straw, 1986). Purchase of pigs within the past 6 weeks appeared more frequently in seropositive than in seronegative herds. Thus, moving infected pigs may have contributed to the spread of PRCV.
Table 3
A cross-sectional study of PRCV in Danish swine herds (1985-1986). Results of a logistic regression model including 316 herds from which data on all variables (listed in Table 2) was ascertained.

| Variable test                                      | \( \beta \) | SE | OR | Wald's \( \chi^2 \) |
|---------------------------------------------------|-------------|----|----|----------------------|
| \( \ln(\text{HPU})^a \)                         | 0.14        | 0.15 | -  | 0.34                 |
| Location in the Jutland peninsula                 | -0.44       | 0.80 | -  | 0.583                |
| Presence of a slurry system                       | -3.33       | 3.25 | 1.92 | 0.05                 |
| Purchase of pigs                                  | 0.65        | 0.33 | -  | 0.001                |
| \( \ln(\text{HPU}) \times \text{location in the Jutland peninsula} \) | 0.93        | 0.28 | -  |                      |
| \( \ln(\text{HPU}) \times \text{presence of a slurry system} \) | 1.39        | 0.94 | -  | 0.14                 |
| Intercept                                         | -1.25       | 0.42 | -  | 0.003                |

\( ^a \text{HPU} = \text{heat-producing units.} \)

Table 4
A cross-sectional study of PRCV in Danish swine herds (1985-1986). Odds ratios (OR) of HPU, for an increase from the first quartile (HPU = 8.5) to the third quartile (HPU = 68.0), with respect to geographic location, and presence or absence of a slurry system, in 316 herds.

| Geographic location         | Jutland peninsula | Island of Funen |
|----------------------------|-------------------|-----------------|
| Slurry system (i.e. slatted floors) | 17.0 (3.3-884) | 24.0 (0.53-1098) |
| No slurry system (i.e. solid floors) | 9.3 (2.9-29.9) | 1.3 (0.73-2.46) |

The detrimental effect of increasing herd size on the incidence and prevalence of swine respiratory disease has frequently been reported (Willeberg et al., 1994). The theoretical curvilinear relationships between herd size and the odds of contracting the disease could also be demonstrated in this study by the use of \( \ln(\text{HPU}) \) or \( \ln(\text{number of pigs}) \) as a herd size measure. The results are in accordance with previous studies of other epizootics with airborne transmission, as demonstrated for Aujeszky's disease (pseudorabies) in Denmark in 1987 to 1988 (Christensen et al., 1990). In relation to geographical location and presence or absence of a slurry system, herd size (\( \ln(\text{HPU}) \)) acted primarily as an effect-modifier rather than a confounding variable (Table 3). Accordingly, further studies are necessary to clarify the herd-size effect. In this case, the geographical density of pigs may be an important

Table 5
A cross-sectional study of PRCV in Danish swine herds (1985-1986). Risk of seropositivity for herds with a seropositive neighbour herd. Results from a sub-set of 125 swine herds with an identified seropositive neighbouring swine herd (logistic multiple regression).

| Logistic regression                        | \( \beta \) | SE | Wald's \( \chi^2 \) test (\( P \)) |
|-------------------------------------------|-------------|----|---------------------|
| \( \ln(\text{HPU of own herd}) \)        | 0.97        | 0.20 | 32.4 (0.000)        |
| Distance to neighbouring seropositive herd in units of 100 m | -0.16      | 0.07 | 6.77 (0.009)        |
aspect, since association between herd density and PRCV infection has previously been reported (Pensaert et al., 1993). Indeed, there was a significant difference between pig density in the sampled area in the Jutland peninsula (430 pigs km$^{-2}$) and on the island of Funen (230 pigs km$^{-2}$) (Landbrugsstatistik, 1987).

Among the above-mentioned risk factors, an increasing herd size and location in the Jutland peninsula support the hypothesis of airborne transmission of PRCV. Also, there was no difference in the prevalence of PRCV between SPF/MS herds and conventional herds, so that rigorous herd security did not seem to protect against PRCV. A hypothesis of airborne transmission was additionally supported by the analysis of herds with an identified neighbouring swine herd, by the finding that the PRCV serological status of neighbouring herds was related, and by the result that closeness of a seropositive herd increased the risk significantly (Table 5). The size of the neighbouring infected herd did not appear to be significantly associated with the risk, but $\beta$ for ln(HPU) of the outcome herd was close to 1. This has been shown by Willeberg et al. (1994) to be the case for infectious diseases at the herd level, provided that the probability of the herd becoming infected ($P$) is related to the probability of one or more animals becoming infected by the equation $P = 1 - (1 - \beta)^n$, where $\beta$ is the probability of one animal becoming infected and $n$ is the number of animals in the herd.

In conclusion, this study has supported the hypothesis of airborne transmission of PRCV in Danish swine herds, but the evidence was based on cross-sectional data. Further insight into the determinants of airborne transmission demands longitudinal data, including meteorological information and exact knowledge of the geographical location of study herds.

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