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## Infectious and rearing-system related risk factors for chronic pleuritis in slaughter pigs

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### Abstract

Chronic pleuritis (CP) in Danish pigs for slaughter is by far the most frequent finding at the routine post-mortem meat inspection. An initial investigation published in 1990 demonstrated infectious and management-related risk factors. Serological testing for additional infectious agents, as well as the need to consider the effect of disease clustering at the herd level, required a re-analysis of the data.

Our re-analysis used a representative sample of 4800 pigs originating from 623 Danish herds. Each pig was examined for the presence of CP and progressive atrophic rhinitis (PAR). The gender of the pig, the weight of the carcass, and the herd of origin were also recorded. Individual blood samples were examined for seropositivity for *Actinobacillus pleuropneumoniae* (AP) serotypes 2, 6, 7, 12, *Haemophilus parasuis*, *Mycoplasma hyopneumoniae* (MYC) and swine influenza (SI). Herd-level information retrieved through a questionnaire included health status, production type, herd size (i.e. pigs per year) and vaccination procedures.

Associations between CP and infectious, individual and herd-related factors were investigated by logistic regression with random effects. Among pigs from herds with conventional health status, seropositivity for AP serotypes 2 and 6, and MYC had odds ratios (ORs) of CP of 9.0, 1.6 and 1.8, respectively. Neither seropositivity for AP serotype 7 nor SI were associated with CP by themselves, but interacted: OR of CP of 5.3 (1.8) when present at the same time among pigs exhibiting (not exhibiting) PAR. An association of PAR with CP was found, and PAR interacted with AP serotype 7:

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OR = 10.0 (4.3) when both factors were present among pigs exposed (non-exposed) to SI. The OR (0.97) for an increase of carcass weight by 1 kg was negligible.

In pigs from specific pathogen-free (SPF) herds, seropositivity for MYC and herd size were associated with CP. Moreover, for a herd size of 1000 pigs, CP was associated with exposure to MYC by an OR of 3.3 (decreasing to 1.9 when the herd size was increased by 1000). Farrow-to-finish as opposed to finishing herd had an OR of CP of 3.2.

In conventional herds, seropositivity for AP serotype 2 and MYC were associated with 51% and 29% of the occurrence of CP. In SPF herds, farrow-to-finish as opposed to finishing herds was associated with 47% of the occurrence of CP. Seropositivity for MYC was associated with 33% (39%) of the occurrence of CP in herds with a size > ( $\leq$ ) 1500 pigs.

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## 1. Introduction

Chronic pleuritis (CP) is a frequent finding in Danish slaughter swine. The prevalence of CP detected at the routine post-mortem meat inspection has been increasing steadily since the beginning of the 1980s. The proportion of pigs with this lesion was 0.14 in 1987 (when the data for the present study were collected)—increasing to 0.25 of the total kill in 2000. CP constitutes about 68% of all lesions detected at slaughter (G. Christensen, 2001, personal communication).

Several infectious agents and environmental factors have been associated with CP (Willeberg et al., 1984; Easterday and Hinshaw, 1992; Nicolet, 1992a,b; Pijoan, 1992; Christensen, 1995).

Associations between occurrence of CP in individual slaughter swine and different microbiological as well as individual and herd-related risk factors were investigated by Mousing et al. (1990). Pleuritic lesions were associated with seropositivity against *Actinobacillus pleuropneumoniae* (AP) serotypes 2 and 6, and swine influenza (SI) sub-type H1N1, as well as progressive atrophic rhinitis (PAR), decreasing carcass weight and increasing herd size.

Due to computational restraints, Mousing et al. (1990) did not account adequately for the fact that the data were from clusters of animals (herds) (McDermott and Schukken, 1994). Mousing et al. (1990) did, however, recognize this problem.

Our aim was to determine the risk of CP associated with infectious, individual and herd-related factors, accounting for disease clustering within herds. Our study was based on data from the investigation by Mousing et al. (1990), but supplemented with serological data on AP serotypes 7 and 12 and *Mycoplasma hyopneumoniae* (MYC). Additionally, the proportion of CP that was attributable to each of the risk factors identified in the random-effects model was measured on the basis of the adjusted odds ratio (OR).

## 2. Materials and methods

The sampling procedure, the data collection and some of the serological methods have been described in detail in the study by Mousing et al. (1990). A brief description follows:

### 2.1. Sampling procedure, data collection and serological methods

The sampling frame was all pigs delivered to a large Danish abattoir, for a 10-week period in the fall of 1987. The sample constituted 240 pigs obtained weekly twice (a total of 4800 pigs from 623 herds for the whole period).

Randomization procedures incorporated weekday and slaughter line (one of three). Every second or third pig on the line was sampled until a total of 240 pigs were obtained.

Pigs were marked and blood sampled in connection with bleeding. Prior to the routine post-mortem meat inspection, carcasses were ad hoc-inspected thoroughly for presence of CP and PAR. Information on gender (castrated male versus female), carcass weight (kg), and herd identification was retrieved through the abattoir database.

Farm owners of sampled herds were asked to fill in a mailed questionnaire. The questionnaire requested information on herd size (the number of pigs produced in the past 12 months), production type (farrow-to-finish versus finishing herd) and vaccination programs against AP serotype 2 and *Haemophilus parasuis* (HPAR). Additional information was requested on health status (conventional versus specific pathogen-free (SPF) herd). SPF herds are declared free of AP serotype 2, MYC, toxin-producing *Pasteurella multocida*, *Brachyspira hyodysenteriae*, *Sarcoptes scabiei* and *Haematopinus suis*. Some serotypes of AP may be accepted within the SPF program, but the health status of a particular herd is changed accordingly e.g. SPF-A (+AP serotype 6). SPF herds infected with MYC are designated “MS herds”.

The serological investigation was carried out in two stages. Initially, sera were examined for presence of antibodies to AP serotypes 2 and 6, HPAR and SI (Mousing et al., 1990). Subsequently, an examination for antibodies to AP serotypes 7 and 12 and MYC was added. An initial screening (A.L. Schirmer, unpublished data) indicating a fairly high prevalence of test-positive pigs for AP serotype 7 (12.4% of 863 sera) and serotype 12 (13.4% of 1037 sera collected from SPF herds) lead to the decision to include these two serotypes in the investigation. Sera (sampled by convenience) from the initial 504 pigs had been used for other purposes unrelated to this study, and they were therefore no longer available for the latter examination.

Sera were examined for seropositivity for AP serotypes 2, 6, 7, 12 and HPAR by a modified complement-fixation test (CFT) (Casey, 1965; Nielsen, 1982). Presence of antibodies to SI sub-type H1N1 (any of the strains 4744 and 6019) was investigated by application of a hemagglutination inhibition (HI) test (Mousing et al., 1990). Presence of antibodies to MYC was examined by application of a modified monoclonal blocking enzyme-linked immunosorbent assay (ELISA) (Feld et al., 1992; Sørensen et al., 1992).

Maximum likelihood estimates of the sensitivity (Se) and specificity (Sp) of the CFT for AP serotype 2 are 90.6 and 98.7% (Enøe et al., 2001). The Se and Sp of the CFT for AP serotypes 6, 7, 12 and HPAR were unknown—but were assumed to be comparable to the Se and Sp of the CFT for AP serotype 2. The Se and Sp of the HI were assumed to be  $\geq 67$  and 100% (Mousing et al., 1990). Estimates of the Se and Sp of the ELISA for MYC are  $\geq 98$  and  $\geq 93\%$  (Sørensen et al., 1997). The fairly high Se and Sp of the serological tests allowed an individual level as well as a herd-level interpretation of test results.

## 2.2. Analytical methods

Rogan–Gladen estimates of true prevalence ( $P$ ) for the infectious risk factors were calculated using the prevalence of seropositive pigs (AP) and the Se and Sp of the diagnostic test by the equation  $P = (AP + Sp - 1) / (Se + Sp - 1)$  (Rogan and Gladen, 1978).

The pig was the unit of analysis. Pigs originating from the same herd were not regarded as mutually independent and were considered to make up a cluster. The intra-class correlation coefficient (ICC) for CP was estimated to evaluate disease clustering within herds. It was assumed that there was at least one infectious agent involved in the development of CP. Only herds with one or more pigs with CP were included. The estimation was done with the variance (ANOVA) estimator for continuous data (Donald and Donner, 1988).

Correlation between carcass weight and herd size was investigated by Spearman's rank correlation coefficient. The associations between presence of CP in the individual pig (the dependent variable) and the various serological (AP serotypes 2, 6, 7, 12, HPAR and MYC), individual (gender, carcass weight and PAR) and managerial factors (production type and herd size) were investigated by logistic regression models with random effects (EGRET Statistical Package Reference Manual, 1993). The models were specified as logistic-binomial regression for 'distinguishable' data (LBDD) models (indicating that each individual pig had its own vector of explanatory covariates). Pigs from herds vaccinating against AP serotype 2 and HPAR ( $n = 406$ ), and pigs having ambiguous or missing results for one or more of the explanatory variables ( $n = 1676$ ) were omitted prior to the analyses. One herd with SPF status that was known to have had an acute outbreak of pneumonia due to AP serotype 2 prior to slaughter also was omitted ( $n = 37$ ). CP was expected to be associated with different risk factors in herds with conventional as opposed to SPF/MS health status; therefore, data were stratified into two sub-sets according to health status of the herds and analyzed separately. In each of the sub-sets, a multivariable LBDD model including all main effects was specified. The model was reduced in a backward-elimination procedure. The variable with the least significance in Wald's test was removed and it was tested whether the loss of information was significant using a likelihood-ratio test at each step. Alpha for removing a variable from the model was 0.1. The scale of the logits for the continuous variables was examined (Hosmer and Lemeshow, 1989) and different transformations were used in order to investigate the linearity assumption. Two-factor interactions between the remaining variables were examined in a backward-elimination procedure similar to the one previously applied. Adjusted ORs and 95% confidence intervals (CIs) were calculated for main effects and two-factor interactions (Hosmer and Lemeshow, 1989).

A likelihood-ratio test statistic (which tested the model with no random-effects parameter against the model with a random-effects parameter) was compared to a one-tailed normal distribution (EGRET Statistical Package Reference Manual, 1993).

To assess the possible bias in inference in the study by Mousing et al. (1990), results from the ordinary logistic regression (OLR) model (Mousing et al., 1990) were compared to results from an LBDD model by the ratios of the coefficients and the standard errors provided by the two models.

The proportion of CP in the population that was attributable to each of the risk factors identified in the LBDD models, respectively, was calculated as the etiologic fraction (EF). An approach described by Bruzzi et al. (1985) for calculation of EF from case-control data (using adjusted regression coefficients from a multivariable logistic-regression model) was adopted for the use in a cross-sectional setting. For each of the categorical factors identified in the multivariable LBDD models, the proportion of the sample exposed to the factor,  $P(E)$ , and the proportion of the sample with CP,  $P(D)$  was obtained along with the estimated adjusted OR. An adjusted estimate of the relative risk (RR) was obtained from  $P(E)$ ,  $P(D)$  and OR (Beaudeau and Fourichon, 1998) and the EF was calculated as  $EF = (P(E)(RR - 1)) / (1 + P(E)(RR - 1))$  (Kleinbaum et al., 1982). The EF was calculated for the two-factor interactions between the categorical variables using the adjusted OR and the marginal distributions for individuals exposed to both factors as opposed to one or none. When one of the variables in the interaction term was continuous, it was dichotomized at the median and the EF of the categorical variable was estimated within each stratum. Note that the EFs do not necessarily add up to 100%. In cases where the adjusted OR was  $<1$ , the prevented fraction (PF) was calculated using the equation  $PF = (P(E)(1 - RR)) / (RR + P(E)(1 - RR))$  (Kleinbaum et al., 1982) in an analogous way. Approximately, 95% CIs for RRs and EFs (PFs) were obtained, by calculating RR and EF (PF) for the upper and lower 95% CIs for the adjusted OR, respectively.

### 3. Results

Serological findings at the pig and herd level are presented in Tables 1–3. Pigs seropositive for MYC were detected in all MS herds ( $n = 22$ )—but also in 13 of the 44 SPF herds. One serotype of AP was detected in 124 (37.5%), two serotypes in 81 (24.5%), three serotypes in 56 (16.9%) and four serotypes in 16 (4.8%) of 331 conventional herds with sufficient serological information (herds vaccinating against AP serotype 2 were not included).

Table 1

Rogan–Gladen estimates of true prevalence ( $P$ , %) of infected pigs within different categories of health status (Denmark, 1987)

| Serological factor | Health status                            |                                   |                                    |
|--------------------|--|-----------------------------------|------------------------------------|
|                    | Conventional ( $n = 3067$ ) <sup>a</sup> | SPF/MS ( $n = 798$ ) <sup>a</sup> | Unknown ( $n = 935$ ) <sup>a</sup> |
| AP serotype 2      | 38                                       | 3 <sup>b</sup>                    | 23                                 |
| AP serotype 6      | 9  | 14                                | 11                                 |
| AP serotype 7      | 27                                       | 2                                 | 22                                 |
| AP serotype 12     | 9  | 39                                | 14                                 |
| HPAR               | 49                                       | 25                                | 42                                 |
| SI                 | 78                                       | 67                                | 70                                 |
| MYC                | 77                                       | 41                                | 62                                 |

<sup>a</sup> Total number including serological samples with missing values.

<sup>b</sup> SPF pigs re-infected with AP serotype 2.

Table 2

Median within herd seroprevalences (% positive pigs) in herds supplying at least one seropositive pig to the sample (Denmark, 1987)

| Serological factor         | Health status |                 |         |
|----------------------------|---------------|-----------------|---------|
|                            | Conventional  | SPF/MS          | Unknown |
| AP serotype 2 <sup>a</sup> | 88            | 73 <sup>b</sup> | 100     |
| AP serotype 6              | 25            | 33              | 50      |
| AP serotype 7              | 43            | 11              | 50      |
| AP serotype 12             | 20            | 33              | 40      |
| HPAR <sup>a</sup>          | 50            | 30              | 50      |
| SI                         | 75            | 50              | 77      |
| MYC                        | 89            | 90              | 82      |

<sup>a</sup> Herds vaccinating against AP serotype 2 and HPAR were not included.

<sup>b</sup> Attributable to one SPF herd re-infected with AP serotype 2.

Sufficient data for analysis was present in the sub-sample of 2064 pigs from 321 herds with a conventional health status. CP was found in 30.3% of the pigs. The median proportion of pigs with CP within the 182 herds with at least one pig with CP was 0.41. The median number of pigs per herd was 4 (min. 1; max. 58). The ICC for CP was 0.21. Results from multivariable LBDD models showed that seropositivity for AP serotypes 2 and 6, and MYC significantly increased the odds of CP (Table 4). Seropositivity for AP serotype 7 or SI was not associated with CP but interacted significantly (increasing the odds of CP when present at the same time) (Table 4). PAR had a positive interaction (marginally significant) with AP serotype 7 (Tables 4 and 5). Carcass weight showed a marginally significant but slightly negative association with CP (Table 4; OR = 0.97, for 1 kg increase).

In the sub-sample of 617 pigs from 63 SPF/MS herds, CP was found in 17.6% of the pigs. The median proportion of pigs with CP within the 37 herds with at least one pig with CP was 0.17. The median number of pigs per herd was 8 (min. 1; max. 30). The ICC for CP was

Table 3

Number and percentage of herds supplying at least one serologically positive pig to the sample, within different categories of health status in 623 herds (Denmark, 1987)

| Serological factor         | Health status                               |    |                                      |    |  |    |
|----------------------------|---|----|--------------------------------------|----|--|----|
|                            | Conventional ( <i>n</i> = 421) <sup>a</sup> |    | SPF/MS ( <i>n</i> = 74) <sup>a</sup> |    | Unknown ( <i>n</i> = 128) <sup>a</sup> |    |
|                            | No.   | %  | No.                                  | %  | No.                                    | %  |
| AP serotype 2 <sup>b</sup> | 128   | 33 | 1 <sup>c</sup>                       | 1  | 35                                     | 27 |
| AP serotype 6              | 120   | 29 | 27                                   | 37 | 28                                     | 22 |
| AP serotype 7              | 201   | 55 | 8                                    | 12 | 49                                     | 41 |
| AP serotype 12             | 130   | 36 | 46                                   | 70 | 29                                     | 24 |
| HPAR <sup>b</sup>          | 300   | 72 | 44                                   | 61 | 82                                     | 64 |
| SI                         | 282   | 67 | 59                                   | 80 | 75                                     | 59 |
| MYC                        | 300   | 83 | 35 <sup>d</sup>                      | 53 | 79                                     | 68 |

<sup>a</sup> Including herds with missing values for serological findings.

<sup>b</sup> Herds vaccinating against AP serotype 2 and HPAR were not included.

<sup>c</sup> SPF herd re-infected with AP serotype 2.

<sup>d</sup> In all of 22 MS herds and 13 of 44 SPF herds, at least one seropositive pig was detected.

Table 4  
Risk factors for CP in slaughter pigs (Denmark, 1987)<sup>a</sup>

| Risk factor                  | <i>b</i>               | S.E. ( <i>b</i> )       | <i>P</i> | OR             | 95% CI, OR | RR             | 95% CI, RR |
|------------------------------|------------------------|-------------------------|----------|----------------|------------|----------------|------------|
| <i>Conventional herds</i>    |                        |                         |          |                |            |                |            |
| AP serotype 2                | 2.20                   | 0.15                    | <0.001   | 9.0            | 6.7, 12.1  | 4.1            | 3.4, 4.9   |
| AP serotype 6                | 0.48                   | 0.21                    | 0.022    | 1.6            | 1.1, 2.4   | 1.4            | 1.0, 1.7   |
| AP serotype 7                | -0.33                  | 0.22                    | 0.145    | - <sup>b</sup> | -          | - <sup>b</sup> | -          |
| MYC                          | 0.59                   | 0.16                    | <0.001   | 1.8            | 1.3, 2.5   | 1.5            | 1.2, 2.0   |
| SI                           | 4.89×10 <sup>-2</sup>  | 0.15                    | 0.748    | -              | -          | -              | -          |
| PAR                          | 0.68                   | 0.28                    | 0.016    | -              | -          | -              | -          |
| (AP serotype 7 × SI)         | 0.85                   | 0.28                    | 0.003    | -              | -          | -              | -          |
| (AP serotype 7 × PAR)        | 1.10                   | 0.57                    | 0.053    | -              | -          | -              | -          |
| Weight (kg)                  | -2.56×10 <sup>-2</sup> | 1.32 × 10 <sup>-2</sup> | 0.052    | - <sup>c</sup> | -          | - <sup>c</sup> | -          |
| <i>SPF/MS herds</i>          |                        |                         |          |                |            |                |            |
| MYC                          | 1.72                   | 0.54                    | 0.002    | - <sup>d</sup> | -          | - <sup>d</sup> | -          |
| Production type <sup>e</sup> | 1.16                   | 0.27                    | <0.001   | 3.2            | 1.9, 5.4   | 2.7            | 1.7, 4.4   |
| Pigs per year                | 6.54×10 <sup>-4</sup>  | 2.52 × 10 <sup>-4</sup> | 0.009    | - <sup>f</sup> | -          | - <sup>f</sup> | -          |
| (MYC × Pigs per year)        | -5.32×10 <sup>-4</sup> | 2.72 × 10 <sup>-4</sup> | 0.051    | -              | -          | -              | -          |

<sup>a</sup> Multivariable logistic-binomial regression on a sub-set of data with sufficient information, including 2064 pigs from 321 conventional herds and 617 pigs from 63 SPF/MS herds. One SPF herd (37 observations) re-infected with AP serotype 2 was not included in the analysis.

<sup>b</sup> Stratum-specific ORs and RRs for main effects involved in interaction terms are shown in tables.

<sup>c</sup> For an increase in carcass weight by 1.0 kg, OR = 0.97.

<sup>d</sup> The OR for MYC depends on the value of pigs per year. For a herd size of 1000 (2000), OR = 3.3 (1.9).

<sup>e</sup> Farrow-to-finish versus finishing herd.

<sup>f</sup> The OR for an increase in pigs per year by 1000 was 1.03 (1.07) among pigs seropositive (seronegative) to MYC.

0.05. Seropositivity for MYC and herd size were associated with CP (Table 4). Moreover, a marginally significant (negative) interaction between MYC and herd size was found; for a herd size of 1000, the MYC OR = 3.27; for 2000 pigs, the MYC OR for CP was 1.92. The odds of CP was increased by 1.03 (1.07) when the output of pigs per year was increased by 1000 among pigs seropositive (seronegative) for MYC. Farrow-to-finish as opposed to finishing herd had OR = 3.2 (Table 4). The likelihood-ratio test statistic for the random effects parameter was significant ( $P < 0.001$ ) in the analysis including conventional herds, when compared to a one-tailed normal distribution (EGRET Statistical Package Reference Manual, 1993).

Results from the OLR model reported by Mousing et al. (1990) were compared to the results from a multivariable LBDD model (by means of the ratios of the coefficients and the standard errors of the coefficients) based on the sub-sample of 3127 pigs from 623 herds with mixed health status, where the factors AP serotypes 2 and 6, SI, PAR, herd size, carcass weight and (carcass weight)<sup>2</sup> were included. The parameter estimates only changed slightly—but the standard errors in the random-effects model increased by a factor of 1.1–1.6. Consequently, (carcass weight)<sup>2</sup> (significant in the OLR model by Wald's statistic) was not significant in the random-effects model. The other factors remained significant ( $P < 0.05$ ) in the random-effects model. The random-effects term was significant ( $P < 0.001$ ) in a likelihood-ratio test (indicating the presence of extra-binomial variation).

Table 5  
Risk factors for CP in slaughter pigs (Denmark, 1987)<sup>a</sup>

| Risk-factor strata <sup>b</sup>                    |                                  | OR   | 95% CI, OR | RR  | 95% CI, RR |
|--|----------------------------------|------|------------|-----|------------|
| Factor 1 (and 2 and 3, if appropriate)             | Factors 2 and 3 (as appropriate) |      |            |     |            |
| AP serotype 7 (+ versus –)                         | PAR (+) and SI (+)               | 5.1  | 1.7, 15.6  | 1.8 | 1.3, 2.2   |
|  | PAR (+) and SI (–)               | 2.2  | 0.7, 6.7   | 1.4 | 0.8, 2.2   |
|  | PAR (–) and SI (+)               | 1.7  | 1.2, 2.4   | 1.4 | 1.1, 1.7   |
|  | PAR (–) and SI (–)               | 0.7  | 0.5, 1.1   | 0.8 | 0.5, 1.1   |
| PAR (+ versus –)                                   | AP serotype 7 (+)                | 5.9  | 2.2, 15.8  | 2.6 | 1.7, 3.4   |
|  | AP serotype 7 (–)                | 2.0  | 1.1, 3.4   | 1.5 | 1.1, 2.0   |
| SI (+ versus –)                                    | AP serotype 7 (+)                | 2.5  | 1.5, 4.0   | 1.9 | 1.4, 2.8   |
|  | AP serotype 7 (–)                | 1.1  | 0.8, 1.4   | 1.0 | 0.8, 1.3   |
| AP serotype 7 and SI (+ + versus – –) <sup>c</sup> | PAR (+)                          | 5.3  | 1.7, 16.5  | 2.0 | 1.3, 2.7   |
|  | PAR (–)                          | 1.8  | 1.2, 2.6   | 1.5 | 1.1, 2.0   |
| AP serotype 7 and PAR (+ + versus – –)             | SI (+)                           | 10.0 | 3.7, 27.4  | 2.5 | 1.9, 2.8   |
|  | SI (–)                           | 4.3  | 1.6, 11.7  | 2.3 | 1.4, 3.1   |
| SI and PAR (+ + versus – –)                        | AP serotype 7 (+)                | 14.6 | 4.7, 44.7  | 4.6 | 2.9, 5.9   |
|  | AP serotype 7 (–)                | 2.1  | 1.1, 3.9   | 1.6 | 1.1, 2.2   |
| AP serotype 7 and SI and PAR (+ + + versus – – –)  | –                                | 10.5 | 4.5, 24.4  | 3.1 | 2.4, 3.5   |

<sup>a</sup> Stratum-specific ORs and RRs for parameters contained in the interaction terms in the logistic-binomial regression model for conventional herds.

<sup>b</sup> Presence (absence) of PAR, seropositivity for AP serotype 7 or SI denoted by + (–).

<sup>c</sup> Joint effect of the interacting parameters.

The EF of MYC in SPF/MS herds was estimated within two different categories of herd size ( $\leq 1500$  or  $>1500$  pigs per year) (Table 6). The PF for AP serotype 7 amounted to 6.6% (95% CI: 0, 16.7%) among pigs from conventional herds.

#### 4. Discussion

Test-positive finishing pigs for MYC were detected in 83% of conventional and in 53% of SPF/MS herds. The median within herd prevalence for MYC was considerable: positive titers were found in  $>82\%$  of the pigs. Seropositive pigs were detected in all MS herds (as expected)—but also in some of the SPF herds (30%). However, these herds might have been re-infected by MYC at the time of sampling (although still maintaining their SPF status) or simply misclassified as SPF herds by the farmers when filling in the questionnaire (when in fact they were MS herds).

Although some cross-protection between AP serotypes has been reported (Nielsen, 1982), two or more serotypes were found to be present simultaneously in many herds (46%). The merits of the SPF production system (in terms of reduced levels of respiratory diseases) is documented (Keller, 1973; Madsen, 1988; Sanker and Gerbola, 1989;

Table 6  
Risk factors for CP in slaughter pigs (Denmark, 1987)<sup>a</sup>

| Risk factor                              | EF (%)         | 95% CI |
|--|----------------|--------|
| <i>Conventional herds</i>                |                |        |
| MYC                                      | 29             | 15, 42 |
| AP serotype 2                            | 51             | 45, 57 |
| AP serotype 6                            | 3              | 0, 6   |
| AP serotype 7                            | 7 <sup>b</sup> | 0, 17  |
| SI                                       | 2              | 0, 1   |
| PAR                                      | 3              | 1, 5   |
| AP serotype 7 and Si <sup>c</sup>        | 9              | 3, 15  |
| AP serotype 7 and PAR                    | 1              | 0, 2   |
| <i>SPF/MS herds</i>                      |                |        |
| MYC (herd size # 1500 pigs) <sup>d</sup> | 39             | 12, 62 |
| MYC (herd size > 1500 pigs)              | 33             | 2, 60  |
| Production type <sup>e</sup>             | 47             | 27, 64 |

<sup>a</sup> Estimated EF for pathological and serological risk factors based on two sub-samples including 2064 pigs from 321 conventional herds and 617 pigs from 63 SPF/MS herds.

<sup>b</sup> PF (OR < 1.0).

<sup>c</sup> Joint effect of the interacting risk factors.

<sup>d</sup> EF for MYC was estimated within two intervals of herd size and are not additive.

<sup>e</sup> Farrow-to-finish versus finishing herd.

Christensen, 1995; Christensen and Enøe, 1999). Our findings showed that test-positive pigs for AP serotypes 2 or 7 were much less-frequently detected in SPF/MS than in conventional herds. AP serotypes 6 and 12 are considered to be of low virulence and are allowed in some herds within the Danish SPF production system. AP serotypes 6 and 12 were detected more frequently in SPF/MS than in conventional herds. Restrictions on infection spread of specific pathogens seem to be efficient within the SPF production system—but when preventive measures are less restrictive, infectious agents may spread easily.

In herds with a conventional health status, CP was associated with seroprevalence of three out of four serotypes of AP. The differences in the increase in odds of CP due to exposure to each of the above serotypes might reflect differences in virulence factors. SI was not in itself associated with CP—but increased the odds of CP in pigs that were seropositive for AP serotype 7. Interactions between these two agents previously were reported by Scatozza and Sidoli (1986) and Bröring et al. (1989). PAR increased the odds of CP by itself (even though the effect was minor)—but the increase was higher among seropositive pigs for AP serotype 7 than in seronegative pigs. It seems likely (from a biological point of view) that turbinate atrophy and introduction of an infection in the respiratory tract are associated. PAR was not identified with a highly sensitive diagnostic method (post-mortem meat inspection). Lack of Se might have weakened the associations between PAR and CP and the other explanatory variables. MYC is usually not believed to be associated with CP because pleuritis is an uncommon lesion in pigs with enzootic pneumonia (Ross, 1992), although this has been reported (Falk and Lium, 1991). In our study, the EF for MYC was 29% mainly due to the high proportion of MYC-positive pigs.

The slight decrease in odds of CP associated with an increase in carcass weight might be an indirect effect of age, because CP resolves with time (Mousing, 1988).

In herds with a SPF/MS health status, farrow-to-finish versus finishing herds had an EF of 47%. This might be explained by the presence of one or more infectious factors unaccounted for in the study (such as *Streptococcus suis*; Reams et al., 1994), if these factors are more common in herds with sows than without sows (sows are reservoirs). That MYC was a risk factor is supported by the difference in the proportions of CP detected at slaughter in pigs from SPF (6.7%) and MS herds (9.8%), respectively (Figures for 1987, G. Christensen, 2001, personal communication). Assuming that the proportion of seropositive pigs for MYC in the sample is representative for the population and that pigs from SPF/MS herds only differ with respect to exposure status for this infection, the effect of seropositivity for MYC in the population was estimated at 19.1% (using population data from the sampling period in 1987). The calculated effect of MYC was larger using the regression estimates than for the population data—but the estimate based on the population figures was contained within the 95% CI of the regression estimates. However, the difference might also be explained by higher Se of the recording of CP in our investigation, compared to the routine post-mortem meat inspection that provides the background for the population data. Part of the increase in risk attributed to MYC may in fact be due to secondary infections unaccounted for in this study (such as *P. multocida*) because MYC interacts with other respiratory infections (Ciprian et al., 1988; Ross, 1992; Yagihashi et al., 1984).

The negative correlation between herd size and odds of CP attributed to MYC could not be easily explained.

Two distinctly different sets of risk factors operated in conventional and SPF/MS herds, respectively, emphasizing the need to analyze data from herds with different health status separately.

We found a fairly high intra-class correlation for CP in conventional herds. In a study of lesions in finishing pigs by Elbers et al. (1992), the coefficient for pleurisy (0.12) was considerably lower than ours (0.21). However, one possible explanation for this difference is that the study by Elbers et al. (1992) was based on data from the routine post-mortem meat inspection. McDermott and Schukken (1994) estimated or presumed ICCs ranging from 0.05 to 0.10 in the study by Mousing et al. (1990). However, ICCs might be higher in intensive production systems and in the presence of highly infectious diseases (conventional versus SPF/MS) as is the case in the fattening pig production. The high ICC we found indicates that disease clustering at the herd level should be accounted for in the risk-factor analysis.

This is confirmed by the re-analysis of the data published by Mousing et al. (1990) because inflated standard errors were found in the LBDD model. McDermott and Schukken (1994) expected that three of the seven variables would turn out non-significant when accounting for clustering. However, only one variable (after an elimination process) was non-significant after adjustment for overdispersion (i.e. (carcass weight)<sup>2</sup>) causing (fortunately) only minor bias in the inferences drawn from the results of the original study. Mousing et al. (1990) discussed possible bias in the inference but they found no reason to abandon their findings, because a validation of their estimates by comparison with estimates derived through an analysis on a sub-set of data (where herd was included as a fixed effect) gave no such indication.

The extent of clustering within herds might vary according to the structure, management and production type of the herds. However, we had no detailed information about this and did not take it into account in the analyses.

Comparisons of the estimated ORs and RRs clearly showed that the rare-disease assumption did not apply in this study. The prevalence of CP was high in pigs from herds with both conventional (30%) and MS/SPF (18%) health status. Consequently, the OR was a biased estimator of the RR. Therefore, both measures should be reported in similar situations.

## 5. Conclusion

Test-positive pigs for MYC were detected in 83% of the herds with conventional health status. Seropositivity for AP serotypes 7 or 12 also was a frequent finding in herds with either health status.

Among pigs from herds with conventional health status, seropositivity for AP serotypes 2 and 6, and MYC increased the odds of CP. Neither seropositivity for AP serotype 7 nor SI increased the odds of CP by themselves, but they had a positive interaction. PAR increased the odds of CP and interacted positively with AP serotype 7. Increasing carcass weight decreased the odds of CP slightly.

In pigs from SPF/MS herds, seropositivity for MYC and herd size increased the odds of CP. Additionally, MYC and herd size interacted negatively. Farrow-to-finish as opposed to finishing herd increased the odds of CP.

Our study and the re-analysis of the initial investigation by [Mousing et al. \(1990\)](#) confirm the need to adjust for extra-binomial variation due to clustering, as pointed out by [McDermott and Schukken \(1994\)](#).

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