

Herd factors associated with the seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from farrow-to-finish pig herds

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Abstract – This cross-sectional epidemiologic study was conducted in 150 randomly selected farrow-to-finish pig herds to investigate descriptive epidemiological characteristics of infections with three different serovars of *Actinobacillus pleuropneumoniae*, and to identify risk factors for the within-herd seroprevalences of these serovars. Different farm characteristics ($n = 28$) were examined as potential risk factors for the percentage of pigs with antibodies against serovars 2, 3 and 9. The presence of antibodies was measured using an indirect ELISA. Logistic regression analyses were used to assess the associations between the potential risk factors and the proportion of seropositive pigs. The median within-herd seroprevalences were 95% (range: 0-100%), 100% (range: 10-100%), and 35% (range: 0-100%) for serovars 2, 3, and 9, respectively. There was a positive association ($P < 0.001$) between each of these serovars. The within-herd seroprevalence of serovar 2 was significantly higher in farms that purchased gilts from ≥ 2 origin herds (OR = 2.33; $P < 0.05$) and in farms with poor biosecurity measures (OR = 4.62; $P < 0.05$). The proportion of pigs seropositive for serovar 3 was significantly higher when tested pigs were slaughtered in May-August and in November-December (OR = 5.96; $P < 0.001$), in herds without a growing unit (OR = 2.63; $P < 0.01$), and in herds with a direct air-entry into the finishing unit (OR = 1.92; $P < 0.05$). The within-herd seroprevalence of serovar 9 increased significantly in herds with poor biosecurity measures (OR = 1.76; $P < 0.05$). The study documented that infections with *A. pleuropneumoniae* serovars 2, 3, and 9 were very common in the selected herds, and that the sero-epidemiological characteristics and risk factors showed some variation depending on the serovar. The purchase policy of gilts and biosecurity measures are risk factors that can be improved fairly easily on pig farms.

swine / *Actinobacillus pleuropneumoniae* / seroprevalence / epidemiology

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Résumé – Facteurs de risque associés à la séroprévalence d’*Actinobacillus pleuropneumoniae* serovars 2, 3 et 9 chez le porc charcutier provenant d’élevages naisseurs-engraisseurs. Une étude transversale séro-épidémiologique vis-à-vis de trois sérovats (2, 3 et 9) d’*Actinobacillus pleuropneumoniae* a été conduite chez des porcs charcutiers à l’abattoir. Ces animaux provenaient de 150 élevages naisseurs-engraisseurs sélectionnés au hasard. Il s’agissait d’identifier les principaux facteurs explicatifs de la variation de la séroprévalence intra-troupeau pour ces trois sérovats. Différentes caractéristiques d’élevages ($n = 28$) ont été étudiées comme étant susceptibles d’expliquer la séroprévalence. La présence d’anticorps a été évaluée par ELISA indirect. Les associations entre ces facteurs ont été calculées par régression logistique. La prévalence médiane intra-élevage d’animaux séroréacteurs était respectivement de 95 % (étendue : 0-100 %), 100 % (étendue : 10-100 %) et 35 % (étendue : 0-100 %) pour les sérovats 2, 3 et 9. Il existe une association positive ($P < 0.001$) entre les trois sérovats. Le pourcentage de porcs séropositifs vis-à-vis du sérovar 2 était plus élevé dans les porcheries où les éleveurs avaient introduit de nouvelles cochettes provenant de plus de 2 élevages ($OR = 2.33$; $P < 0.05$) et dans les porcheries avec des mesures de biosécurité insuffisantes ($OR = 4.62$; $P < 0.05$). La prévalence du sérovar 3 était plus élevée lorsque les porcs étaient abattus entre mai et août d’une part et de novembre à décembre d’autre part ($OR = 5.96$; $P < 0.001$), dans les élevages sans unité de pré-engraissement ($OR = 2.63$; $P < 0.01$) ainsi que dans les élevages avec une entrée d’air directe ($OR = 1.92$; $P < 0.05$). La détection d’animaux séropositifs pour le sérovar 9 augmentait dans les élevages avec des mesures de biosécurité insuffisantes ($OR = 1.76$; $P < 0.05$). Cette étude a démontré que les infections à *A. pleuropneumoniae* étaient fréquentes dans les élevages sélectionnés et que les facteurs de risque étaient différents selon le sérovar impliqué. L’introduction de cochettes dans le troupeau et les mesures de biosécurité sont des facteurs de risque qui peuvent être facilement améliorés dans des élevages porcins.

porc / *Actinobacillus pleuropneumoniae* / séroprévalence / épidémiologie

1. INTRODUCTION

Porcine pleuropneumonia is a contagious respiratory disease caused by the gram-negative bacterium *Actinobacillus pleuropneumoniae*. The disease is distributed worldwide and causes severe economic losses to the pig industry. The clinical course of the disease can vary widely, ranging from the acute forms with severe clinical signs and a high mortality to the more chronic forms with few or even without any clinical symptoms.

Because antibiotic therapy is effective only during the initial phases of the disease [7], control measures rather than treatment of clinically affected animals should be pursued. The control can be accomplished in different ways namely by the use of antimicrobials, by vaccination, and by improvements of management practices and housing conditions [31]. Continuous or intermittent use of antimicrobials during the fattening period should not be instituted over a long time because of the increased risk for antibi-

otic resistance [33] and the presence of antibiotic residues in the slaughter pigs. Strategic medication appears to work very well in some herds but it can only be used when the periods of risk are known and when outbreaks can be predicted accurately. A wide range of vaccines against pleuropneumonia has been developed. The most recent ones generally provide protection against the disease but like the use of antimicrobials, they cannot prevent colonisation of *A. pleuropneumoniae* organisms in the respiratory tract nor eliminate the infection from a herd. Improvement of management practices and housing conditions are considered to be very effective in reducing the risk for clinical outbreaks and economic consequences of pleuropneumonia [11]. These measures however are usually discussed in a general way.

Many recommendations concerning the control of pleuropneumonia are based on clinical experience or derive from knowledge of other respiratory diseases. In this respect, there is insufficient epidemiologic

knowledge about specific risk factors associated with *A. pleuropneumoniae* infections in swine farms. Some studies focused on descriptive sero-epidemiologic characteristics during the grow-finishing period [2], on the interaction with other respiratory pathogens [9, 34], or on the lung lesions associated with *A. pleuropneumoniae* infections [23]. Besides identifying different farm characteristics as risk factors, it is also important to quantify the impact of each risk factor. A cross-sectional multivariate study conducted in a large number of pig herds constitutes a very useful tool to accomplish this purpose. The outcome of such studies can provide very interesting information and assist in implementing specific and cost-effective control measures on infected swine farms.

The present study was conducted to investigate descriptive epidemiological characteristics of infections with three different serovars of *A. pleuropneumoniae*, and to identify risk factors for the within-herd seroprevalences of these serovars in 150 farrow-to-finish (FTF) pig herds. Serovars 2, 3 and 9 were included because preliminary data of diagnostic laboratories indicated that these serovars were frequently isolated from Belgian pigs.

2. MATERIALS AND METHODS

2.1. Study population and herd factors

The population of this cross-sectional study comprised 150 FTF pig herds with more than 50 sows. The herds were randomly selected from all FTF pig herds located in West-Flanders and in an adjacent small region of East-Flanders. This is an area with a surface of 11% of Belgium, and in which 54% of the Belgian pig population is located.

Each herd was visited once to collect different farm characteristics as potential risk factors for respiratory disease. Therefore, we used a questionnaire with precise defi-

nitions of the data to be recorded. The data were obtained through inspections of the pigs and the pig units, and through face-to-face interviews of the pig farmers. Information pertained to herd size, month of slaughter, pig and pig herd density in the municipality, type of breed of the sows, management practices, housing conditions, disease prevention procedures, hygienic measures within and outside the unit (biosecurity measures). Management practices included purchase policy of gilts, stocking densities in the finishing units, number of pigs per pen and per compartment, and type of production. Housing conditions referred to presence of a growing unit in which pigs are raised from approximately 70 until 120 days of age, compartmentalisation, number of compartments in the finishing unit, type of ventilation system and type of floor in the finishing unit. All these data were contemporary for the pigs that were examined at slaughter. More details about the study population and the different potential risk factors are described in a previous paper [17]. Vaccination against *A. pleuropneumoniae* was not practised in any of the selected swine farms.

2.2. Slaughterhouse inspection and serological testing

From each herd, one group of 60 to 150 pigs was sent to the slaughterhouse, and a blood sample was taken from 25 pigs per herd. Pigs were selected systematically for blood sampling, and 20 out of the 25 samples were tested for the presence of serum antibodies against *A. pleuropneumoniae* serovars 2, 3 and 9. The sampling procedure permitted to detect with 95% certainty at least one positive pig from a group of 150 pigs when the minimum within-herd prevalence was 14 %.

An indirect ELISA, based on the description by Trottier et al. [32], was used for detection of antibodies against heat-stable antigens of *A. pleuropneumoniae* serovars 2,

3 and 9. Heat-stable antigens were prepared by boiling a suspension of 10^{10} CFU/mL of the *A. pleuropneumoniae* strains for 1 hour. After centrifugation, the supernatant was filtered through a 0.45 μ m filter (International Medical, Brussels, Belgium). Wells of a microtiter plate (Nunc-Immuno Plate MaxiSorp™, GIBCO, Ghent, Belgium) were coated with 100 μ L of each antigen (1.7 μ g/mL). Plates were incubated with 1/400 dilution of sera. Bound antibodies were detected using 1/250 diluted peroxidase-conjugated rabbit anti-pig serum (Sigma Chemical Co, St Louis, Mo, USA). Positive controls consisted of sera from 2 pigs experimentally infected with *A. pleuropneumoniae* serovars 2, 3 or 9. The cut-off value was determined using 44 known negative sera and was expressed as the mean absorbance value (at 450 nm) + 3 times the standard deviation. All infected pigs were tested positive whereas all non-infected animals remained negative. No or very minimal cross-reactions were observed with other serovars. Consequently, the ELISA tests used in the present study are considered to be sensitive and specific.

2.3. Statistical analyses

The 95% confidence intervals of the within-herd seroprevalences of the three *A. pleuropneumoniae* serovars were calculated as described by Daniel [6]. Associations between the seroprevalences of the different serovars were tested at the animal-level using logistic regression analysis (SAS 6.12, Glimmix) [29]. The herd was included as a random effect [16]. Associations between the seroprevalences of two different serovars were expressed by the odds ratio (OR) and they were analysed in two directions, with either agent as the dependent and independent variable.

The farm characteristics were examined as potential risk factors for the percentage of pigs with antibodies against *A. pleuropneumoniae* serovars 2, 3 and 9. Therefore, logis-

tic regression analyses (SAS 6.12, Proc Genmod) [29] were performed at the herd-level with the proportion of seropositive pigs being the dependent variable, and the different herd factors being the independent variables. Overdispersion due to the non-independence between pigs of the same herd [19] was taken into account by including a scale parameter in the logistic regression model [5]. A forward stepwise procedure was used to select independent variables that were significantly associated with the different seroprevalences [24]. The goodness of fit of the final models was assessed by calculating the root mean squared errors (RMSE) using the following formula:

$$\left[\sqrt{\frac{1}{n} \sum (P_{\text{observed}} - P_{\text{fitted}})^2} \right],$$

with n denoting the number of herds, and P denoting the within-herd seroprevalence [20]. Potential values for RMSE range between 0% and 100%, corresponding to perfect predictability or complete lack of predictability by the model, respectively. Odds ratios and their 95% confidence intervals were calculated from the final logistic regression models. More details about the statistical analyses are explained in a previous paper [17].

3. RESULTS

The median within-herd seroprevalences were 95% (range: 0-100%), 100% (range: 10-100%), and 35% (range: 0-100%) for serovars 2, 3, and 9, respectively (Tab. I). The distributions of the within-herd seroprevalences of serovar 2 and 3 were both skewed with a longer tail towards the lower seroprevalences (left skewed), whereas the within-herd seroprevalence of serovar 9 was skewed with a longer tail towards the higher seroprevalences (right skewed) (Fig. 1). The farms showed a fairly high degree of variation in the percentage of seropositive pigs per farm, especially for serovar 9. In only one herd, all the tested pigs were

Table I. Within-herd seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from 150 farrow-to-finish pig herds.

	Within-herd seroprevalences		
	Serovar 2	Serovar 3	Serovar 9
Minimum	0 [0-14] ^a	10 [2-29]	0 [0-14]
Median	95 [78-100]	100 [86-100]	35 [14-59]
Mean	81 [59-93]	90 [71-98]	39 [20-63]
Maximum	100 [86-100]	100 [86-100]	100 [86-100]

^a Between brackets: 95% confidence intervals.

Table II. Significant associations ($P < 0.001$), expressed in odds ratios, between the seroprevalences of *A. pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from 150 farrow-to-finish pig herds.

Dependent variable	Independent variable		
	Serovar 2	Serovar 3	Serovar 9
Serovar 2	–	8.81 [6.10-12.74] ^a	4.25 [3.27-5.53]
Serovar 3	9.43 [6.87-12.9]	–	3.46 [2.60-4.62]
Serovar 9	3.83 [2.86-5.13]	3.33 [2.31-4.79]	–

^a Between brackets: 95% confidence intervals.

seronegative for serovar 2 and in another herd, all tested pigs were seronegative for serovar 9. All herds were seropositive for *A. pleuropneumoniae* serovar 3. The percentages of herds in which all investigated pigs tested positive for the different serovars were 40%, 57%, and 1% for serovars 2, 3, and 9, respectively. Pigs seropositive for serovar 9 had a 4.25 times higher risk to be seropositive for serovar 2 and a 3.46 times higher risk for being seropositive for serovar 3. Pigs seropositive for serovar 3 had 8.81 times higher risk to be seropositive for serovar 2 (Tab. II). The outcomes were similar when the analyses were performed in the opposite directions i.e. with the prevalence of either serovar as dependent or independent variable.

The risk factors for the within-herd seroprevalences of *A. pleuropneumoniae* serovars 2, 3 and 9 are presented in Table III. The within-herd seroprevalence of serovar 2

was significantly higher in farms that purchased gilts from ≥ 2 herds (OR = 2.33), and in herds with poor biosecurity measures (OR = 4.62). The proportion of pigs seropositive for serovar 3 was significantly higher when tested pigs were slaughtered in May-August and in November-December (OR = 5.96), in farms without a growing unit (OR = $1/0.38 = 2.63$), and in farms with a direct air-entry ventilation system in the fattening unit (OR = 1.92). The within-herd seroprevalences of serovar 3 in the bi-monthly periods were: J-F 84%, M-A 81%, M-J 97%, J-A 96%, S-O 87%, N-D 97%. The within-herd seroprevalence of serovar 9 increased significantly in herds with poor biosecurity measures (OR = 1.76). The interaction terms and the squared terms of the continuous independent variables in the intermediate and final models were not significant. RMSE values for the final models were 25%, 17% and 22% for serovars 2, 3, and 9, respectively.

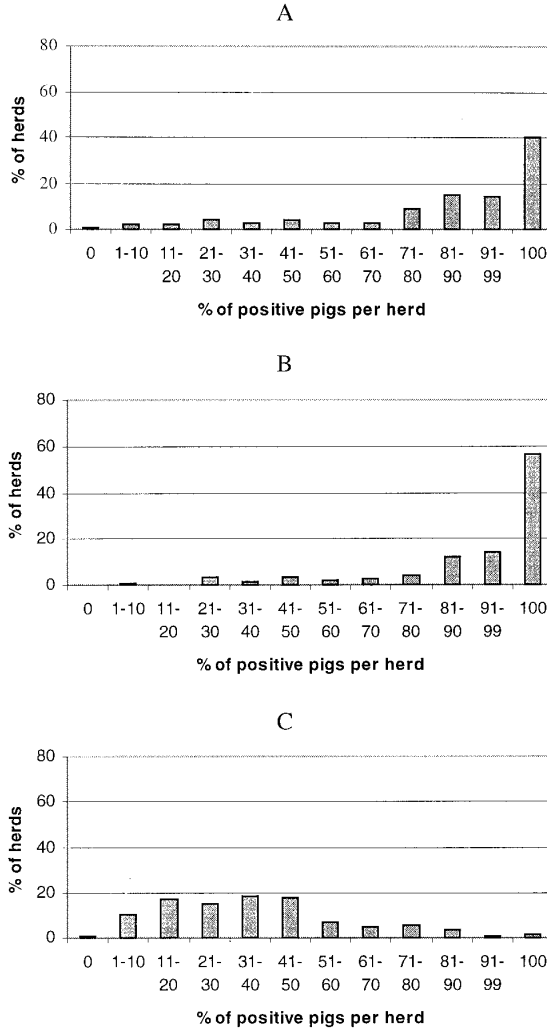


Figure 1. The distributions of the within-herd seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2 (A), 3 (B) and 9 (C) in 150 randomly selected farrow-to-finish pig herds.

4. DISCUSSION

In this cross-sectional study, different sero-epidemiological characteristics of infections with *A. pleuropneumoniae* in slaughter pigs from Belgian farrow-to-finish pig farms were investigated. Since the farms were selected randomly, the results may be

generalized to other Belgian farrow-to-finish swine farms located in regions with a high pig density. It appeared that almost all herds were infected with each of the three serovars. The seroprevalences were particularly high for serovars 2 and 3, and to a lesser extent for serovar 9. Elbers et al. [8] also found higher seroprevalences for

Table III. Risk factors for the within-herd seroprevalences of *Actinobacillus pleuropneumoniae* serovars 2, 3 and 9 in slaughter pigs from 150 farrow-to-finish pig herds: parameters in the final logistic regression models, coefficients with standard errors, *P*-values and odds ratios with 95% confidence intervals (CI).

<i>Parameters</i>	<i>Coefficients ± standard error</i>	<i>P-value</i>	<i>Odds ratio [95% CI]</i>
<i>Final model for A. pleuropneumoniae serovar 2 (deviance 1194; RMSE 25%)</i>			
Intercept	0.8494 ± 0.3208	0.008	–
Purchase of gilts			
≥2 herds	0.8475 ± 0.3840	0.027	2.33 [1.10-4.95]
from one herd	–0.0790 ± 0.3619	0.827	0.92 [0.45-1.88]
no purchase	0.0000 ± 0.0000	–	–
Biosecurity measures			
poor	1.5303 ± 0.6330	0.016	4.62 [1.34-15.9]
moderate	0.3947 ± 0.3450	0.253	1.48 [0.75-2.92]
good	0.0000 ± 0.0000	–	–
Scale parameter	2.9146 ± 0.0000	–	–
<i>Final model for A. pleuropneumoniae serovar 3 (deviance 694; RMSE 17%)</i>			
Intercept	2.7519 ± 0.3878	< 0.001	–
Month of slaughter			
May-Aug and Nov-Dec	1.7857 ± 0.3942	< 0.001	5.96 [2.75-12.91]
other months	0.0000 ± 0.0000	–	–
Presence of growing unit			
yes	–0.9736 ± 0.3428	0.005	0.38 [0.19-0.74]
no	0.0000 ± 0.0000	–	–
Ventilation system			
direct air-entry	0.6527 ± 0.2953	0.027	1.92 [1.08-3.43]
indirect air-entry	0.0000 ± 0.0000	–	–
Scale parameter	2.2687 ± 0.0000	–	–
<i>Final model for A. pleuropneumoniae serovar 9 (deviance 675; RMSE 22%)</i>			
Intercept	–0.8079 ± 0.2032	< 0.001	–
Biosecurity measures			
poor	0.5651 ± 0.2776	0.0418	1.76 [1.02-3.03]
moderate	0.3753 ± 0.2240	0.0938	1.46 [0.94-2.26]
good	0.0000 ± 0.0000	–	–
Scale parameter	2.0562 ± 0.0000	–	–

serovar 2 (55%) than for serovar 9 (4%) in slaughter pigs from herds in the Southern part of the Netherlands. Our results further corroborate with other studies [4, 10, 12, 35] showing that multiple serovars may exist within the same farm. In contrast to a Danish study [2], we did not observe farms to be infected with one dominant serovar of *A. pleuropneumoniae*. Serovars 2, 3 and 9

were considered in the present study because preliminary information from diagnostic laboratories indicated that these serovars were frequently isolated from Belgian pigs. Although each of the three serovars may be implicated in clinical outbreaks of porcine pleuropneumonia ([18], Hoflack et al., unpublished results), the serologic data provided convincing evidence that most swine

farms with intensive rearing systems are subclinically infected with *A. pleuropneumoniae*. According to Mousing et al. [23] and Beskow et al. [3], infections with serovar 2 are a very important cause of chronic pleuritis in slaughter pigs from Scandinavian countries.

Positive associations were observed between each pair of the investigated serovars. It is not possible to explain the exact cause of these associations since many factors may be involved. The positive associations may be due to the high seroprevalences and consequently, to the high number of pigs that was simultaneously positive for the different serovars. It is unlikely that cross-reactions in the serological tests account for the positive associations, since the ELISA tests used have a high specificity. Cross-reactions are more common between serovars 1, 9 and 11; serovars 3, 6 and 8, and serovars 4 and 7 because of structural similarities in some epitopes of the lipopolysaccharides in these serovars [26]. The positive associations may result from similar management practices or environmental conditions promoting the spread of these serovars. Some evidence for this hypothesis was obtained for the association between serovar 2 and 9 since poor biosecurity measures was a common risk factor for both serovars. However, no evidence was available to explain the other positive associations. It is unlikely that infection with one serovar may predispose to infection with another serovar, since infection with one serovar can induce partial cross-protection against infection with another serovar [13]. The slightly different ORs for the associations between 2 serovars (Tab. II) are caused by different variations in within-herd seroprevalences with respect to these serovars.

A significantly higher proportion of slaughter pigs with antibodies against serovar 2 was observed in herds that purchased gilts from ≥ 2 origin farms. There was however no difference between herds with on-farm raising of gilts and farms that purchased the gilts from one origin farm.

The higher risk to be seropositive for serovar 2 in herds that purchased gilts from ≥ 2 origin farms may be attributed to the increased risk of introducing the pathogen by subclinically infected (carrier) gilts. Although we have not investigated the gilts directly, it is reasonable to assume that many of them have been exposed previously to *A. pleuropneumoniae* infections [15] and that they have become carriers of the organism. Carrier pigs usually harbor the bacteria in the tonsils and/or in necrotic lung lesions, less frequently in the nasal cavity [14]. Carrier gilts may shed *A. pleuropneumoniae* organisms to other pigs in the herd, including their offspring [18]. The results further demonstrated that poor biosecurity measures significantly increased the risk of seropositivity for infections with serovar 2 (OR = 4.62) and 9 (OR = 1.76). The variable biosecurity measures in the present study pertained to prevention of disease entry by trailers carrying pigs, feed or manure, presence of a sanitary room, use of farm specific boots, clothes and protective head-gear. The variable was classified as being good, moderate or bad when these measures were always, sometimes or never practiced in the herd. Biosecurity issues are becoming more important in the modern swine industry, especially in farms with a high health status [1]. Unfortunately, only a few studies have assessed the importance of biosecurity measures in swine farms to prevent *A. pleuropneumoniae* infections.

The farm characteristics identified as risk factors for the within-herd seroprevalences of *A. pleuropneumoniae* serovar 2 were different from those associated with serovar 3. Comparative studies on the pathogenesis of these *A. pleuropneumoniae* serovars may elucidate mechanisms that could explain the observed differences in risk factors. There was some seasonal pattern in the seroprevalence of serovar 3 with more than 5 times higher risks for seropositivity for pigs slaughtered in May through August and in November and December. It is not clear why the risk to be seropositive at slaughter

was higher in these periods, especially for the pigs slaughtered during the summer months. The possibility that the worst herds were selected during these periods cannot be ruled out but this is unlikely since the herds were selected and investigated at random. It was shown that pigs raised in herds with a growing unit were at lower risk (OR = 0.38) to be positive for serovar 3. In a growing unit, pigs are raised from approximately 70 days (when they leave the nursery unit) until 120 days of age (when they are moved to the finishing unit). The presence of a growing unit has the advantage that younger pigs do not share the same airspace with older finishing pigs. Horizontal transmission of serovar 3 organisms may have been lower in these herds or the infection may have been delayed to a later time in the finishing unit, resulting in a lower seroprevalence at slaughter age. The risk to be seropositive for serovar 3 was almost twice as high in herds with a direct air-inlet in the fattening unit. Such a ventilation system allows the outside air to enter the units and the pig area directly without being warmed up first. Consequently, such a system will more likely be associated with wider variations in temperature, relative humidity and other climatic parameters in the pig units. These factors are generally considered to be important in the development of porcine pleuropneumonia [31]. Thus, the climatic conditions were probably better or remained more constant in the finishing units with an indirect air-entry.

The within-herd seroprevalence of slaughter pigs was used to assess the presence and spread of three serovars of *A. pleuropneumoniae* in the herds. Measuring the presence of antibodies, like most other tests currently available for porcine pleuropneumonia, is not a perfect tool to detect infection in living pigs. It has been shown that *A. pleuropneumoniae* organisms can remain undetected in some seropositive pigs, whereas some seronegative pigs can be infected [21, 30]. However, serologic data of slaughter pigs may be useful to assess the

infection status of the herds with regard to porcine pleuropneumonia. Recording clinical disease or lung lesions at slaughter is less informative for this purpose. Measuring clinical disease is less useful because in most endemically infected herds, subclinical infections rather than clinical outbreaks are the rule [11]. In addition, precise measurements of clinical respiratory disease are difficult to obtain and in many instances, multiple pathogens are involved. Focusing on slaughter lesions has the disadvantage that no etiological diagnosis can be established and that the results only reflect the end-stage of a particular pathological process. Consequently, lung lesions resulting from *A. pleuropneumoniae* infections occurring early in the fattening period, may be healed at slaughter age [22]. Using serology, clinical as well as subclinical infections can be detected [25]. Clinical outbreaks of pleuropneumonia cause substantial economic losses to the pig industry, especially because of the high mortality. However, the economic losses incurred by pig producers due to subclinical infections are also very high. According to Rohrbach et al. [27], seropositive, but subclinically infected pigs require 5.6 additional days to reach market weight.

A cross-sectional design was used in this study. This implies that the risk factor and the disease outcome namely the proportion of seropositive pigs in each herd were measured at one point in time [28]. The significant risk factors may not be considered as being causal factors but they should be interpreted as factors that contribute significantly to a higher seroprevalence in the herds. The odds ratios in this study quantify the increased or decreased risk of finding a seropositive pig in a herd when the risk factor is present in this herd. More discussion about the strengths and weaknesses of a cross-sectional study design has been presented in the first paper [17]. Because we used serology as disease parameter, the risk factors are not necessarily the same as those associated with clinical disease or with lung lesions caused by *A. pleuropneumoniae*.

The risk factors associated with the percentage of seropositive pigs within the herds may be related with the introduction of pathogens into the herd, with the spread of pathogens within a herd, or with decreased resistance of the pigs against infection. It is not possible however, in this study, to assess the importance of each of these three mechanisms. The RMSE values of the final logistic regression models appeared to be fairly low, ranging from 17 to 25%. These values correspond to a moderate to high predictability of the final models [20].

In conclusion, this study documented that epidemiologic characteristics like overall seroprevalence, distribution of within-herd seroprevalence among farms and risk factors associated with the within-herd seroprevalence showed some variation across the three serovars. Some risk factors like the purchase policy of gilts and biosecurity measures can be improved on the farms fairly easily, whereas others like seasonal influences and adverse housing characteristics are much more difficult to change. Additional research, preferably including a higher number of different serovars and using longitudinal studies, is warranted to further investigate the epidemiological characteristics of *A. pleuropneumoniae* infections in pig herds.

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