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## Transmission of *Actinobacillus pleuropneumoniae* among weaned piglets on endemically infected farms



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### ABSTRACT

Clinical outbreaks due to *Actinobacillus pleuropneumoniae* occur recurrently, despite the wide-scale use of antimicrobials or vaccination. Therefore, new approaches for the prevention and control of these outbreaks are necessary. For the development of alternative measures, more insight into the transmission of the bacterium on farms is necessary. The aim of this cohort study was to quantify transmission of *A. pleuropneumoniae* amongst weaned piglets on farms. We investigated three possible transmission routes: (i) indirect transmission by infected piglets within the same compartment, (ii) transmission by infected pigs in adjacent pens and (iii) transmission by direct contact within pens. Additionally, we evaluated the effect of independent litter characteristics on the probability of infection. Two farms participated in our study. Serum and tonsil brush samples were collected from sows pre-farrowing. Serum was analysed for antibodies against Apx toxins and Omp. Subsequently, tonsil brush samples were collected from all piglets from these dams ( $N = 542$ ) in three cohorts, 3 days before weaning and 6 weeks later. Tonsil samples were analysed by qPCR for the presence of the *apxIVA* gene of *A. pleuropneumoniae*. Before weaning, 25% of the piglets tested positive; 6 weeks later 47% tested positive. Regression and stochastic transmission models were used to assess the contribution of each of the three transmission routes and to estimate transmission rates. Transmission between piglets in adjacent pens did not differ significantly from that between non-adjacent pens. The transmission rate across pens was estimated to be  $0.0058 \text{ day}^{-1}$  (95% CI: 0.0030–0.010), whereas the transmission rate within pens was ten times higher  $0.059 \text{ day}^{-1}$  (95% CI: 0.048–0.072). Subsequently, the effects of parity and serological response of the dam and litter age at weaning on the probability of infection of pigs were evaluated by including these into the regression model. A higher dam ApxII antibody level was associated with a lower probability of infection of the pig after weaning; age at weaning was associated with a higher probability of infection of the pig after weaning. Finally, transmission rate estimates were used in a scenario study in which the litters within a compartment were mixed across pens at weaning instead of raising litter mates together in a pen. The results showed that the proportion of infected piglets increased to 69% if litters were mixed at weaning, indicating that farm management measures may affect spread of *A. pleuropneumoniae*.

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## 1. Introduction

One of the most common pathogenic bacteria in the pig industry is *Actinobacillus pleuropneumoniae*. *A. pleuropneumoniae* can infect pigs of all ages and can cause pneumonia, pleuritis, growth retardation and mortality (Gottschalk and Taylor, 2006). Up to now, most control measures have focused on prevention of clinical signs by improving hygiene and housing conditions, the application of antimicrobials or vaccination. An alternative approach for control, which focuses on preventing colonisation and reduction of bacterial transmission instead of reduction of clinical signs, might result in more sustainable pig farming without the abundant use of antimicrobials. Vaccination seems to have been unable to curb transmission (Velthuis et al., 2003) and attempts for elimination of *A. pleuropneumoniae* from farms by application of antimicrobials have often shown to be unsuccessful (Lariviere et al., 1990; Hunneman and Oving, 1991; Christiansen and Szancer, 2006; Gjestvang et al., 2008). It is therefore not clear whether and how an appropriate control strategy, resulting in prevention of transmission, may be achieved in current pig husbandry.

To develop a strategy that results in reduced transmission it is essential to know at which moment infection occurs and what the route of transmission is. The first likely moment of contracting the bacterium is during the suckling period as piglets can acquire *A. pleuropneumoniae* from the dam (Vigre et al., 2002). In a previous study, we showed that the proportion of *A. pleuropneumoniae* infected pigs at time of weaning was approximately 30% and clustered at litter level (Tobias et al., 2014). For piglets free from *A. pleuropneumoniae* at the time of weaning, it is not clear when they become infected, but at time of slaughter the prevalence may nearly be 100% (Maes et al., 2001; Chiers et al., 2002; Tobias et al., 2012).

Experimentally, it has been shown that transmission of *A. pleuropneumoniae* from subclinically infected pigs by direct contact occurs with a rate  $\beta$  of about  $0.1 \text{ day}^{-1}$ , meaning that every infectious pigs can infect on average 0.1 susceptible piglets per day (Velthuis et al., 2002, 2003). Transmission by indirect contact, e.g. by air, has been observed experimentally as well (Torremorell et al., 1997; Jobert et al., 2000; Kristensen et al., 2004), but the quantitative importance of both routes on farms is unclear.

The aim of this study was to identify and quantify transmission routes for *A. pleuropneumoniae* in weaned pigs on endemically infected farms. Comparison was made between the rates to become infected with *A. pleuropneumoniae* due to (i) indirect contact with infected pigs in the same compartment (between-pen transmission), due to (ii) direct and indirect contact with infected pigs in adjacent pens (adjacent pen transmission) or due to (iii) direct contact with infected pen mates (within-pen transmission). We further assessed whether dam characteristics (parity and antibody titres) and litter age at weaning affected the risk of infection after weaning. Finally, transmission rates were estimated for the relevant transmission routes. These estimates were subsequently used in simulation models to evaluate the effect of mixing litters after weaning on the transmission of *A. pleuropneumoniae*.

## 2. Materials and methods

### 2.1. Study design

A cohort study was performed on two *A. pleuropneumoniae* serovar 2 endemically infected farrow-to-finish farms (A (1700 sows) and B (760 sows)) in The Netherlands. On farm A (1700 sows) all pigs were finished on site, whereas on farm B (760 sows) 70% of the pigs was sold around 10 weeks of age around 24 kg live weight and the rest was finished on site. Farms were selected based on the ability to comply with the research protocol, raising their own breeding stock, the confirmed presence of *A. pleuropneumoniae* infection, absence of preventive group treatments with antimicrobials after weaning and no vaccination of sows or pigs against *A. pleuropneumoniae* in the last year. See for further details Tobias et al. (2014). Four cohorts were composed of randomly selected sows, stratified by parity, from four farrowing groups of sows. Within each cohort, sows were assigned randomly to a farrowing crate.

For follow-up after weaning, litters in which pigs were cross-fostered (three litters per cohort) were excluded. In Farm A we selected the twelve litters with the lowest prevalence for each cohort, to maximise on the population at risk. For logistical reasons, this was not possible on farm B, and therefore sixteen litters per cohort from farm B were randomly selected. All selected litters were randomly assigned to a pen in the nursery using computer aided lottery method. Litters were moved to the nursery compartment litter by litter to prevent contact between litters during transport. If litter size at weaning exceeded the maximum number of pigs that was allowed in the nursery pen, the farmer moved some piglets to another nursery compartment and these were excluded for follow-up. In cohort 1 of farm A, piglets were housed in a different compartment than was agreed upon. This resulted in the inclusion of four additional pens which contained piglets of multiple litters from the same cohort. For evaluation of the transmission routes a covariate for these mixed pens was included and for evaluation of associated dam characteristics these litters were excluded.

During the study, cohort 2 of farm A had been treated orally with antimicrobials until 13 days before sampling, because of clinical signs presumably caused by *Streptococcus suis*. The inclusion criteria were not met anymore and this cohort was excluded for follow-up.

During the suckling period a minimal animal movement protocol was applied and during sampling a strict hygiene protocol was applied to prevent transmission caused by personnel. At all sampling moments disposable gloves and boot covers were changed before entering a new pen. Farm workers were instructed to clean their boots before entering a pen when performing health checks or chores. Farmers were blinded for sampling results.

In both farms, compartments for weaning pigs consisted of two rows of pens with a corridor in between. For transmission analyses an adjacent pen was considered a pen that is adjacent to the other within the same row. Some pens had only one adjacent pen, most had two. In farm A two adjacent pens shared a feeding trough on one side; pens were separated by half closed and half wired fences above the

feeding trough, and by wired fences at the opposite site. In farm B pen partition consisted of completely closed fences of  $\pm 50$  cm high. Piglets were fed liquid feed in farm A and creep feed in farm B. The ventilation system in farm A was ceiling ventilation, farm B had ground channel ventilation.

## 2.2. Ethics

The samples were obtained in compliance with Dutch Law on Animal Experimentation and the Act on the Practice of Veterinary Medicine. Informed consent was obtained from the participating farmers.

## 2.3. Sampling and processing of results

Serum samples of sows were obtained 3 weeks before farrowing and analysed by ELISA for antibodies against ApxI, ApxII, ApxIII (Nielsen et al., 2000) and Omp (Kobisch and van den Bosch, 1992). Formal test characteristics for use of samples from sows are not available for these tests. ApxI–III ELISA tests, using recombinant produced toxin antigen rather than purified toxins obtained from *A. pleuropneumoniae*, were reported to have diagnostic sensitivities of 1.0, 0.95 and 0.93 respectively, and a diagnostic specificity of 0.96 (Shin et al., 2011). Antibody titres were obtained on a  $\log_2$  scale, with a maximum of 14; for association analyses with the probability of infection results were coded to “high” or “low”, based on the observed median for the sows in this study. For detailed results of the serum analyses we refer to Tobias et al. (2014). For association analyses dam parity was coded into “low” (parity 1–3) and “high” (parity 4–9), based on the observed median in this study.

All pigs were sampled 3 days before weaning and 42 days later by tonsil brush. Tonsils were brushed with a soft sterile toothbrush for 10 seconds and subsequently samples were processed for analysis by qPCR for the *apxIVA* gene (Tobias et al., 2012) (reported test sensitivity = 0.98 (95% CI: 0.92; 1.0) and specificity of 1.0 (95% CI: 0.96; 1.0)). QPCR was carried out as described previously (Tobias et al., 2014); results were denoted as positive or negative.

In the end piglets were identified according to one of six status: NO or PO, if the piglet tested negative or positive, respectively, at 3.5 weeks, and the piglet was not present at 9.5 weeks; NN or PN, if tested negative or positive, respectively, at 3.5 weeks, and negative at 9.5 weeks; NP or PP, if tested negative or positive, respectively, at 3.5 weeks, and positive at 9.5 weeks. For litters the same classification system was used (LNN, LPN, LNP and LPP), with a negative litter defined as all piglets tested negative and a positive litter as containing any positively tested piglet.

## 2.4. Transmission analyses

In Supplementary Files 1 and 2, the data and the code for the transmission analyses are provided, respectively. In the analysis, three transmission routes were considered:

- i. Transmission within the compartment irrespective of distance between pigs, e.g. airborne transmission.

- ii. Transmission in the same or adjacent pens, e.g. indirectly by large droplets.
- iii. Transmission within the pen, e.g. by direct contacts.

For the transmission analyses, pigs identified as NO or PO were not included, as most had been removed within 2 weeks after weaning. For each pen  $i$ , data consisted of the following variables:

- $S_i$ : the number of initially susceptible pigs (pigs identified as NN or NP).
- $C_i$ : the number of new cases during the observation period (pigs identified as NP).
- $I_i$ : the number of initially infectious pigs (pigs identified as PP) in the same pen. Thirteen PN pigs were not considered infectious, an assumption which we addressed in a sensitivity analysis (see below).
- $J_i$ : the total number of infectious pigs (PP) in adjacent pens as well as the same pen.
- $K_i$ : the total number of infectious pigs (PP) in the entire compartment, including those in the same and adjacent pens.
- $LNP_i$ : an variable indicating new cases in initially infection-free pen (1 if  $I_i = 0$  and  $C_i > 0$ , otherwise 0).
- A binary variable indicating if the litter was mixed (4 pens in cohort 1 of farm A).
- Binary variables indicating dam characteristics: dam parity, ApxI titre, ApxII titre, ApxIII titre, Omp titre.
- Litter age at weaning (between 15 and 30 days).

Transmission was analysed in three steps. In the first step, a grouped regression model was used to assess the contribution of the three transmission routes. Based on the conclusion that only the indirect and within-pen transmission routes were significant, in the second step, a dynamic S-I type transmission model was used to estimate per day within-pen and between-pen transmission rates. In step 3, the regression model of step 1 was extended to include dam factors.

Step 1: regression models details.

For pen  $i$ , the mean probability for a pig to become infected was expressed as

$$p_i = 1 - \left(1 - p_{\text{pen}}(\mathbf{X}_i, \boldsymbol{\theta}_{\text{pen}})\right)^{I_i} \times \left(1 - p_{\text{nb}}(\mathbf{X}_i, \boldsymbol{\theta}_{\text{nb}})\right)^{J_i} \times \left(1 - p_{\text{comp}}(\mathbf{X}_i, \boldsymbol{\theta}_{\text{comp}})\right)^{K_i}$$

with  $p_{\text{pen}}$ ,  $p_{\text{nb}}$ , and  $p_{\text{comp}}$  being the probabilities to become infected by the three transmission routes,  $I_i$ ,  $J_i$ , and  $K_i$  being the numbers of infected pigs as defined above,  $\mathbf{X}_i$  denoting pen-specific covariates, and  $\boldsymbol{\theta}$  denoting the parameters. In step 1 of the analysis, the pen-specific covariates are cohort and mixed pen for the within-pen transmission ( $p_{\text{pen}}$ ) and the cohort only for  $p_{\text{nb}}$ , and  $p_{\text{comp}}$ . To link  $p_i$  with the data, we assumed that the number of new cases  $C_i$  was beta binomially distributed, with sample size  $S_i$ , mean probability  $p_i$  and shape parameter  $\rho$ . The pens that started negative but became positive ( $LNP_i = 1$ ) were treated separately. In these pens  $I_i = 0$ , which means that the model above would attribute all cases to introduction from outside the pen which will result in overestimation of

neighbourhood or compartmental transmission if within-pen transmission route was much more important than the other two routes. Therefore, for these pens the probability  $p_i^{\text{LNP}}$  of having at least one case was included into the likelihood:

$$p_i^{\text{LNP}} = 1 - (1 - p_{\text{nb}})^{J_i S_i} (1 - p_{\text{comp}})^{K_i S_i}$$

Thus, the log-likelihood equation reads

$$l = \sum (1 - \text{LNP}_i) \log [\delta(C_i | p_i, \rho)] + \text{LNP}_i \log p_i^{\text{LNP}},$$

in which  $\delta(\cdot)$  is the density of the beta binomial distribution. This equation was maximised to obtain the parameter estimates.

Step 2: transmission models details.

In step 2, we estimated the transmission rates for within-pen and compartmental transmission. For within-pen transmission, pigs were assumed to become infected by rate  $\beta I/N$ , where  $I/N$  is the prevalence within the pen, and  $\beta$  the transmission rate parameter, defined as the number of new infections caused by one infectious pig per day in a susceptible pen. Compartmental transmission was assumed to be only relevant for introduction into an infection-free pen: pigs were assumed to become infected by rate  $\iota K/N_{\text{comp}}$ , where  $K/N_{\text{comp}}$  is the initial prevalence in the compartment, and  $\iota$  is the between-pen transmission rate parameter, defined as  $\beta$ , but on compartment level. As described in more detail by van Bunnik et al. (2012), it is possible to formulate the above model in terms of differential equations for the probabilities  $q_{[S_i, I_i, I_i + C_i]}(t)$  to have  $I_i + C_i$  infected pigs at time  $t$ , given that the pen started with  $S_i$  susceptible and  $I_i$  infected pigs. These state probabilities (or “Master Equations”) can be solved analytically (van Bunnik et al., 2012), to obtain explicit expressions for the probabilities  $q_{[S_i, I_i, I_i + C_i]}(t = 42)$  of observing  $C_i$  cases at day 42, as in the data. These are combined in the log-likelihood function

$$l_2(\beta, \iota) = \sum \log q_{[S_i, I_i, I_i + C_i]}(t = 42)$$

By this method, the whole transmission chain is accounted for, i.e. new cases are not only attributed to infectious pigs at 3.5 weeks of age as in step 1.

Four different models were compared, with equal or different values for  $\beta$  and  $\iota$  in the three compartments, by means of AIC. Step 2 was performed first on the same data as in step 1, i.e. for pens with  $\text{LNP}_i = 1$  the probability to observe more than one case was included, to enable comparison with the regression model by AIC; and second on complete data, i.e. for pens with  $\text{LNP}_i = 1$  the probability to observe exactly  $C_i$  cases was included, so as to obtain more precise estimates of  $\beta$ .

Step 3: dam factors.

In step 3, the pen-specific covariates  $\mathbf{X}_i$  in the equation for  $p_i$  (step 1) included the various dam factors, by having the probabilities  $p_{\text{pen}}$ ,  $p_{\text{nb}}$ , and  $p_{\text{comp}}$  depend linearly on the dam factors through a logit transform. Data of six pens with mixed litters or inconclusive dam ApxII ELISA results were excluded. Because susceptibility will affect  $p_{\text{pen}}$ ,  $p_{\text{nb}}$ , and  $p_{\text{comp}}$  in the same way, all factors were given a single parameter for all transmission routes. As in step 1, all

possible parameter combinations were compared by AIC. For the final model we checked for collinearity between explanatory litter characteristics by assessing their mutual correlation (by Wilcoxon rank sum test) and their effects on parameter estimates by leaving out explanatory variables one by one.

Statistical analyses were performed with R, version 2.15.1 (R Development Core Team, 2012) and additional packages bbmle, Matrix and VGAM. In all analyses statistical models were compared based on model fit by AIC (Burnham and Anderson, 2002). Confidence intervals for the final models were obtained by profile-likelihood.

## 2.5. Sensitivity analysis

The assumption that PN tested piglets did not contribute to infectivity, was challenged by repeating step 1 assuming that PN tested piglets were infectious.

## 2.6. Simulation of the effect of mixing litters after weaning

To facilitate extrapolation of the results to the field, we evaluated the proportion of positive piglets per cohort if piglets had been randomly allocated to pens after weaning. The total number of infected pigs after 42 days was simulated stochastically using the transmission model as described in step 2 above, with initial conditions, the number of susceptible (NN+NP), infected but not infectious (PN) piglets and infectious (PP) pigs as in the data and the obtained transmission rates in step 2 (see above). This procedure was repeated 1000 times and the mean proportion of positive piglets and range between 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles was returned and compared with the observed cumulative incidence in this study with separately raised litters.

In a second scenario the effect of mixing on the number of infected pigs 42 days later was studied for a virtual cohort of 176 pigs with 10 infectious pigs in 2 pens at weaning which were considered to be either raised separately per litter or being randomly mixed across pens.

## 3. Results

### 3.1. Sampling results

555 piglets in three cohorts (48 litters in three cohorts) were examined after weaning. Thirteen piglets died (PO) of which eleven within 2 weeks after weaning. Twenty-four of 48 litters were free from *A. pleuropneumoniae* infection before weaning (LNP+LNN). In total, 408 of 542 surviving piglets tested negative before weaning and were considered susceptible in the transmission analyses. In total 121 piglets tested positive twice and were considered infectious (PP) (Table 1) with on average 5.0 (range 1–13) infectious pigs per pen. Thirteen infected piglets tested positive at first sampling round and negative 6 weeks later (PN). In the subsequent 6 weeks, 11/24 pens became infected (LNP) with an average of 2.0 (range: 1–6) positive piglets per pen. 120 Newly infected cases were found, resulting in a total of 254/542 (47%) infected piglets at

**Table 1**  
Counts of individual piglet and pen infection status per cohort.

Status of piglets (and pens)	Farm A Cohort 1	Farm B Cohort 1	Farm B Cohort 2	Total
Pigs initially moved to weaning unit (number of pens)	175 (16)	188 (16)	192 (16)	555 (48)
Pigs died between sampling moments (P0 + N0)	1	6	6	13
Pigs susceptible (3.5 weeks; NP + NN)	126	133	149	408
Escaped infection (9.5 weeks; NN)	74	89	125	288
New case (9.5 weeks; NP)	52	44	24	120
(LNP)	(4)	(3)	(4)	(11)
Pigs infectious (3.5 and 9.5 weeks; PP)	43	47	31	134
(LPP)	(10)	(9)	(5)	(24)
Tested negative (9.5 weeks; PN)	5	2	6	13
(number of pens containing PN pigs)	(4)	(2)	(3)	(9)
Pigs infected (9.5 weeks; PP + PN + NP)	100	93	61	254
(LPP + LNP)	(14)	(12)	(9)	(35)
Proportion infectious pigs (3.5 weeks; PP)	0.25	0.26	0.17	0.22
Proportion positive pigs (3.5 weeks; PP + PN)	0.28	0.27	0.20	0.25
Proportion positive pigs (9.5 weeks; PP + PN + NP)	0.57	0.51	0.33	0.47

9.5 weeks of age (Table 1 and Supplementary Fig. 1). Pens with all pigs free from *A. pleuropneumoniae* at 9.5 weeks were not spatially clustered as they were often found next to high prevalence pens (Supplementary Fig. 1).

### 3.2. Transmission analyses

#### Step 1: transmission routes.

In step 1 the model with lowest AIC was a beta binomial regression model with  $p_{\text{pen}} = 0.32$  (95% CI: 0.22; 0.42) and  $p_{\text{comp}} = 0.0015$  (95% CI: 0.0008; 0.0026) for all cohorts (Table 2). The probability  $p_{\text{pen}}$  was not significantly affected by the four mixed pens. Including  $p_{\text{nb}}$  in the model resulted in an estimate of  $p_{\text{nb}} = 0.00$  (95% CI: 0–0.044).

#### Step 2: transmission rates.

In step 2 a SI transmission model was fitted. This was done first with exactly the same data as in step 1, showing both the regression model and the SI models fit equally well. Second, more detailed data were used to obtain final estimates for the within-pen transmission rate  $\beta = 0.059 \text{ day}^{-1}$  (95% CI: 0.048; 0.072) and the rate for

indirect between-pen transmission rate  $\iota = 0.0058 \text{ day}^{-1}$  (95% CI: 0.0030; 0.0010). Direct transmission was 10.3 (95% CI: 5.6–20.1) times as efficient as indirect transmission across pens.

#### Step 3: litter characteristics.

In step 3 dam factors and age at weaning were included in the regression model as used in step 1, but on a subset of the data. The model that fitted the data best (simplest and low AIC) was a model with a binomial distribution, one estimate for  $p_{\text{comp}}$  as well as for  $p_{\text{pen}}$  and additionally age at weaning and dam ApxII titre as model parameters (Table 3). A high ApxII antibody titre of the dam before farrowing was associated with a lower probability of infection for the piglets (OR = 0.34 (95% CI: 0.21; 0.47) after weaning. Pigs weaned at a later age had a higher risk of becoming infected (OR 1.37  $\text{day}^{-1}$  (95% CI: 1.33; 1.42) (Supplementary Table 1). There was no correlation between age at weaning and dam ApxII ( $P = 0.56$ ), and parameter estimates changed less than 34% when litter characteristics were left out one by one, without affecting the directions of the effects.

**Table 2**

Model evaluation for assessing the importance of transmission routes (steps 1 and 2). Model parameters and model fit (AIC) are shown for models of steps 1 and 2 for the simplest, most complicated and best fitting models ranked on AIC of models with a beta binomial distribution. The best fitting model for the data observed is printed in bold.  $k$  = total number of parameters; in beta binomial models  $\rho$  (of the beta distribution) is an additional model parameter. – = not included, X = included, (X) = two additional cohort specific covariates included, (–) no cohort specific covariates included,  $p_{\text{comp}}$  = probability of infection due to compartmental between-pen transmission,  $p_{\text{nb}}$  = probability of infection due to transmission from adjacent pens,  $p_{\text{pen}}$  = probability of infection due to within-pen transmission, n.e. = not evaluated.

Model	$p_{\text{comp}}$ (cohort specific)	$p_{\text{nb}}$ (cohort specific)	$p_{\text{pen}}$ (cohort specific)	Mixing of litters	$\rho$	$k$	AIC	
							Beta binomial model	Transmission model
<b>Model 1 (best fit)</b>	<b>X (–)</b>	–	<b>X (–)</b>	–	<b>X</b>	<b>3</b>	<b>111.0</b>	<b>111.6</b>
Model 2	X (–)	–	X (X)	–	X	5	112.6	113.1
Model 3	X (–)	–	X (–)	X	X	4	113.0	n.e.
Model 4	X (–)	X (–)	X (–)	–	X	4	113.0	n.e.
Model 5	X (X)	–	X (–)	–	X	5	114.0	114.6
Model 6	X (X)	–	X (X)	–	X	7	115.4	116.2
Full model	X (X)	X (X)	X (X)	X	X	11	123.3	n.e.
Model 7	–	X (–)	X (–)	–	X	3	166.0	n.e.
Empty model	X (–)	–	–	–	X	2	181.3	n.e.

**Table 3**

Model evaluation for assessing the importance of transmission routes with inclusion of litter characteristics (step 3). Model parameters and model fit (AIC) are shown for the simplest, most complicated, best fitting model and all models within 2 points of AIC for analyses in step 3. Models which included  $p_{nb}$  had an AIC > 2 points higher than the best model and were not included in this table. The best fitting model for the data observed is printed in bold.  $k$  = total number of explanatory variables,  $\rho$  ( $\rho$  of the beta binomial distribution) is an additional explanatory variables. – = not included, X = included, (–) no cohort specific covariates included, (X) = two additional cohort specific covariates included.

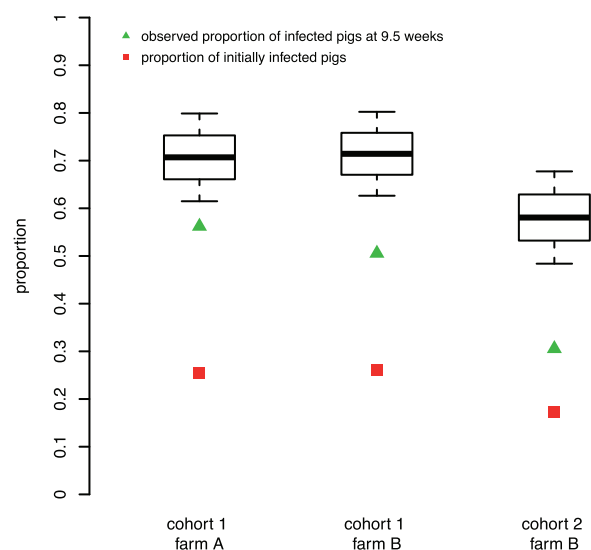
Model	$p_{comp}$ (cohort specific)	$p_{pen}$ (cohort specific)	Ap <sub>xl</sub>	Ap <sub>xII</sub>	Ap <sub>xIII</sub>	Omp	Parity	Age	$\rho$	AIC	$k$
Model 1	X (–)	X (X)	X	X	X	X	–	X	–	73.6	9
<b>Model 2 (best fit)</b>	<b>X (–)</b>	<b>X (–)</b>	–	<b>X</b>	–	–	–	<b>X</b>	–	<b>74.0</b>	<b>4</b>
Model 3	X (–)	X (X)	X	X	–	X	–	X	–	74.1	8
Model 4	X (–)	X (X)	X	X	–	–	–	X	–	74.4	7
Model 5	X (–)	X (X)	X	X	–	–	X	X	–	74.8	8
Model 6	X (–)	X (–)	–	X	–	X	–	X	–	74.9	5
Model 7	X (–)	X (X)	X	X	X	X	X	X	–	75.1	10
Model 8	X (–)	X (–)	X	X	–	–	–	X	–	75.4	5
Model 9	X (–)	X (–)	–	X	–	–	–	X	X	75.4	5
Model 10	X (–)	X (X)	X	X	X	X	–	X	X	75.6	10
Full binomial	X (X)	X (X)	X	X	X	X	X	X	–	77.3	12
Full beta binomial	X (X)	X (X)	X	X	X	X	X	X	X	79.3	13
Empty beta binomial	X (–)	X (–)	–	–	–	–	–	–	–	83.9	2
Empty binomial	X (–)	X (–)	–	–	–	–	–	–	X	89.5	3

### 3.3. Sensitivity analysis

Inclusion of the pigs with status PN as infectious (step 1) had a major impact on  $p_{pen}$  (resulting in three cohort specific estimates for  $p_{pen}$ ), but with a final model AIC of 14.7 points higher, indicating a poorer model fit.

### 3.4. Simulation of the effect of mixing litters after weaning

When piglets had been randomly allocated across pens after weaning instead of being kept together with their litter mates, the results of the simulation showed that the prevalence at compartment level would be approximately 69% instead of 47%, the observed average percentage in this study (Table 4 and Fig. 1).



**Fig. 1.** Cumulative proportion of infected pigs. As observed at 3.5 and 9.5 weeks (PP + NP + PN) and obtained by simulation of randomly allocating piglets to pens after weaning.

The effect of mixing was also evaluated for a virtual cohort of 176 pigs in 16 pens in which two pens contained five infectious pigs. The simulated percentage of infected piglets 6 weeks later was 14% (95% percentiles: 10%; 21%) when litters were not mixed, but 26% (95% percentiles: 18%; 37%) when litters were mixed.

## 4. Discussion

In this study we aimed to quantify the contribution of direct and indirect transmission of *A. pleuropneumoniae* in weaned piglets. Transmission of *A. pleuropneumoniae* between weaned piglets across pens did not differ significantly between adjacent and non-adjacent pens, but the direct transmission rate (within a pen) was ten times higher than across pens. The transmission rate within pens was 0.059 day<sup>-1</sup> (95% CI: 0.048–0.072), which corresponded with earlier results found for *A. pleuropneumoniae* serovar 9 ( $\beta = 0.15$  day<sup>-1</sup> (95% CI: 0.00; 0.61) (Velthuis et al., 2002) and  $\beta = 0.10$  day<sup>-1</sup> (95% CI: 0.033; 0.32) (Velthuis et al., 2003)). It was also found that the dam Ap<sub>xII</sub> antibody level and litter age at weaning were significantly associated with the probability of infection after weaning.

No additional risk of infection from adjacent pens was found, although the upper value of the confidence interval showed that increased transmission between adjacent pens could not be ruled out completely. Other studies showed that airborne transmission of *A. pleuropneumoniae* between neighbouring pens occurred over short distances (1–2.5 m) (Jobert et al., 2000; Kristensen et al., 2004). In these studies, an artificial flow was created between the two pens located at a fixed distance, whereas we studied indirect transmission between pigs in adjacent and non-adjacent pens under field conditions, independent of distance. So, the airborne transmission in these studies may have been due to the same (airborne) mechanism as in our study.

The direct transmission rate was approximately ten times higher than the indirect transmission rate. This order of magnitude has also been found for *Streptococcus suis*

**Table 4**

Proportion of infected pigs 42 days after weaning is determined by management strategy. For the three observed cohorts in this study, as well as a virtual cohort of 176 pigs the observed or simulated proportion of infected pigs is presented. The proportions are calculated as follows  $((PP + PN + NP)/N)$ . For the virtual cohort it was assumed that 176 pigs were housed in 16 pens of which two pens contained 5 infectious pigs at weaning ( $I/N=0.06$ ). The proportion of infected pigs at 6 weeks later is presented while raising the litters separated or randomly mixed after weaning (with 95% percentiles).

	Farm A Cohort 1	Farm B Cohort 1	Farm B Cohort 2	Virtual cohort Simulated proportion
Non-mixing <i>observed</i>	0.57	0.51	0.33	0.14 (0.10; 0.21)
Random mixing at weaning <i>simulated</i> (95% percentiles)	0.70 (0.61; 0.80)	0.71 (0.63; 0.80)	0.58 (0.48; 0.68)	0.26 (0.18; 0.37)

(Dekker et al., 2013) and several viral pathogens (e.g. Classical Swine Fever virus (Klinkenberg et al., 2002), Foot-and-Mouth disease virus (Eblé et al., 2006) and Porcine circovirus (PCV2) (Andraud et al., 2008)). The absolute transmission rates of these pathogens were, however, 5–144 times higher than the rates found in our study, suggesting that control of *A. pleuropneumoniae* transmission by separating uninfected pigs from infected ones may be an option to reduce colonisation prevalence, even under field conditions. Whether it is effective during clinical outbreaks would be highly speculative as the estimated transmission rates do not reflect a situation with clinically diseased pigs.

At first a beta binomial distribution was used for the number of new infections per pen, with  $\rho$  explaining part of between-pen variation. In step 1 of this study (regression model without sow factors), this choice is supported by the better fit of models with a beta binomial probability distribution. However, in step 3, where the effect of dam factors were considered, regression models with a binomial probability distribution fitted the data better. This demonstrates that a few litter related factors explained most of the variation on pen level. Dam ApxII titre was negatively and age at weaning was positively associated with the probability of infection of pigs after weaning.

In our study, one dam factor and age at weaning explained the probability of infection for a pig between 3.5 and 9.5 weeks of age, whereas in a previous study no dam factors were found to explain the probability of infection of piglets between 0 and 3.5 weeks of age despite the apparent between-sow variation in infectivity (Tobias et al., 2014). It may be that between 0 and 3.5 weeks, the unexplained variation in infectivity between sows caused so much variation between litters that more subtle differences in susceptibility could not be observed, whereas in the present study with the sows removed, the effects of maternal immunity and age at weaning could be discerned.

Cruijssen et al. (1995) showed that piglets are protected by maternal antibodies against the development of clinical signs until approximately 12 weeks of age. In line with their results, we also observed that offspring of dams with a high ApxII antibody titre had a lower probability to become infected after weaning than piglets from dams with low titres. However, vaccination studies with Apx toxins (Velthuis et al., 2003) did not induce protection against infection. Possibly, the ApxII antibody titre itself is not the biological explanation for the protective effect, but just reflects a different protective factor. The level of maternal immunity in the pig at a certain time point depends on (a) the level immunity of the dam (Sjölund et al., 2011), (b) the initial amount that was taken up (Vigre et al., 2003) and (c) time, because the half-life of antibodies to

*A. pleuropneumoniae* is approximately 2 weeks (range 1–3 weeks) (Vigre et al., 2003). This may explain why despite lack of correlation between ApxII titre and age at weaning, slight collinearity was observed between final model parameters (step 3). However, as the direction of the effects was not altered if one of the parameters was left out, we concluded that both parameters could be retained in the model.

Results of simulation of mixing litters at weaning emphasises the effects of farm management practices on the dispersion of *A. pleuropneumoniae*. With an initial proportion of 0.25 infected pigs, mixing of litters would result in a low number of pens with all piglets free from *A. pleuropneumoniae*. Therefore mixing litters will result in more piglets exposed to within-pen transmission of *A. pleuropneumoniae*. Consequently, the prevalence 6 weeks later will be higher than when litters are raised separately (Fig. 1). The same will apply for farms in which pen sizes are increased to contain multiple litters, which is considered to be economically more beneficial. The results of the simulation study in which transmission started with 10 infectious pigs in two pens in a virtual cohort of 176 pigs demonstrated that control of transmission is even more effective when the infection process in the suckling phase could be reduced. However, whether and how this could be achieved in commercial pig farms needs to be determined.

## 5. Conclusion

On farms the transmission rate of *A. pleuropneumoniae* between weaned piglets across pens did not differ significantly between adjacent and non-adjacent pens, but the rate within a pen was ten times higher than across pens. Dam factors, such as antibody levels ante partum, were associated with the risk of infection for piglets after weaning.

## Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.prevetmed.2014.07.017>.

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