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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 day^{-1} (95% CI: 0.0030–0.010), whereas the transmission rate within pens was ten times higher 0.059 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 β of about 0.1 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