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A cohort study on *Actinobacillus pleuropneumoniae* colonisation in suckling piglets

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ABSTRACT

Actinobacillus pleuropneumoniae causes respiratory disease in pigs and despite the use of preventive measures such as vaccination and antimicrobials clinical outbreaks still occur. At weaning often many piglets are not colonised. If differences in prevalence between litters are large and if factors were known that could explain these differences, this may provide an opportunity to raise groups of *A. pleuropneumoniae* free piglets. To this end, a cohort study was performed on two endemically infected farrow-to-finish farms. Seventy-six of 133 sows were selected using stratified random selection by parity. Farmers complied with a strict hygiene and animal management protocol to prevent transmission between litters. Tonsil brush and serum samples taken three weeks before parturition were tested for antigen with an *apxIVA* qPCR and antibodies with Apx and Omp ELISAs, respectively. Three days before weaning tonsil brush samples from all piglets ($n = 871$) were collected and tested for antigen. Whereas all sows tested positive both in serology tests as well as qPCR, 0.41 of the litters tested fully negative and 0.73 of all piglets tested negative. The proportion of positively tested piglets in positive litters ranged from 0.08–1.0 (median = 0.36). A grouped logistic regression model with a beta binomial distribution of the probability for piglets to become infected was fitted to the data and associations with explanatory variables were explored. To test the possibility that alternatively the clustering was caused by onwards transmission among the piglets, a transmission model was fitted to the data incorporating sow-piglet and piglet-piglet transmission, but this model did not fit better. The results of this study showed that the number of colonised suckling piglets was highly clustered and mainly attributable to the variability of infectiousness of the dam, but no dam related risk factor for colonisation status of litter or piglets within litters could be identified.

1. Introduction

One of the most common bacterial infections on pig farms is caused by *Actinobacillus pleuropneumoniae*. Infections may often run a subclinical course, but can also result in clinical outbreaks, characterised by pleuritis, respiratory distress, and mortality (Gottschalk and Taylor, 2006). Control and prevention of clinical disease is currently achieved by use of antimicrobials (Mengelers et al., 2000; Hoflack et al., 2001), or vaccination (Tumamao et al., 2004). However, the use of antimicrobials does not clear *A. pleuropneumoniae* from tonsils of colonised pigs

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(Angen et al., 2008) and vaccination does not prevent colonisation of susceptible pigs (Velthuis et al., 2003). Therefore, these measures will not attribute to elimination of *A. pleuropneumoniae* from farms.

Piglets can already become colonised during the suckling stage (Vigre et al., 2002). However, despite the finding that the prevalence of *A. pleuropneumoniae* infections in sows was generally high (Chiers et al., 2002; Fablet et al., 2011; Sjölund et al., 2011), the prevalence of colonised piglets around the time of weaning was found to be rather low under controlled (Vigre et al., 2002) as well as under field conditions (Chiers et al., 2002). In these studies, no information was provided on the distribution of colonisation across litters. Clustering across litters may be expected if some dams transmit the infection more easily than others, and/or if the piglets transmit the bacterium amongst themselves. Understanding the factors that explain variation in colonisation could provide an opportunity to develop control measures on farms.

Differences between litters due to transmission by the dam could be expected if there is variation in maternally derived antibodies, which is related to the level of antibodies in the serum of the dam (Sjölund et al., 2011). It has been reported that these antibodies prevent the development of clinical signs (Cruijsen et al., 1995a), as disease is usually seen after the age at which maternal antibodies have waned (Cruijsen et al., 1995b). Whether antibodies protect pigs against colonisation of *A. pleuropneumoniae* is unknown, but convalescent serum of naturally infected pigs can inhibit bacterial cell adhesion to buccal epithelial cells in vitro (Hamer-Barrera et al., 2004). In turn, parity can affect the level of immunoglobulin passed on from dam to the piglets, but reports are contradictory on this matter. For example, Klobasa and Butler (1987) reported a positive association between parity and concentrations of immunoglobulin in milk, whereas Sjölund et al. (2011) found higher *A. pleuropneumoniae* antibody levels in serum of parity-one sows and Cruijsen et al. (1995b) reported higher antibody titres in offspring from younger sows. These observations raise the question whether characteristics of the dam (parity and antibody level) could be related to the probability that their piglets become colonised and whether sow characteristics can be identified as risk or protective factors.

The objectives of this study were to study clustering of colonisation status of suckling piglets just before weaning in endemically *A. pleuropneumoniae* infected pig farms, and to determine the association between dam parity, antibody levels and the probability of colonisation of litters and piglets within litters during the suckling period.

2. Materials and methods

A cohort study was performed on two farrow-to-finish farms (A and B) in two farrowing rooms (cohorts) per farm. Sows were examined for the presence of *A. pleuropneumoniae* infection by collection of blood and tonsil brush samples approximately three weeks before parturition. The proportions of colonisation at litter and individual piglet level were determined three days before weaning and associations with dam parity and sow serum and brush sample results were evaluated.

2.1. Setting

Two Dutch farrow-to-finish farms (farms A and B) were selected, both privately owned. The farms were selected for the following reasons. Both farms had a history of a high pleuritis and respiratory signs caused by *A. pleuropneumoniae* in finisher pigs. Furthermore, the farms had to raise their own breeding stock, had not used *A. pleuropneumoniae* vaccines in the year before the trial started and did not apply preventive antimicrobial batch treatment during suckling or after weaning. The farms were not related to each other. Finally, the willingness of the farmer and the ability on the farm to comply with the research protocol were the most important criteria for inclusion.

The study population consisted of a census sample of four cohorts with 76 sows and their offspring; 871 piglets. Each cohort was raised within one farrowing room. Samples from farm A were collected from April to June 2011 and samples from Farm B were collected from May to July 2012.

Farm A was a farrow-to-finish farm with 1700 sows; farm B housed 760 sows and 70% of the piglets were sold at 10 weeks of age, the rest was finished on site. Gestating sows in farm A were housed individually or in groups of five animals, whereas in farm B gestating sows were housed in groups of approximately 54 sows. Confirmation of *A. pleuropneumoniae* infections was obtained by performing necropsy on diseased pigs (Gottschalk and Taylor, 2006) on farm A, and serology in growers and finishers on farm B. Additionally, from both farms an *A. pleuropneumoniae* serovar 2 isolate was recovered from affected lung tissue obtained at slaughterhouse investigations after the study period.

2.2. Study population within farms

In both farms, two farrowing week batches were selected based on the farms' schedules to be able to comply with the hygiene and management protocol. A total of 133 F1 sows, bred for finishing, were sampled three weeks before parturition and all tested positive in qPCR. From the four farrowing batches four study cohorts of sows were composed by computer aided stratified random selection. Stratification was performed on parity, relative to the parity distribution in the farrowing batch. On farm A, two cohorts of 18 sows were randomly selected from 38 and 26 sows originating from two farrowing week batches, two weeks apart, respectively. On farm B, two cohorts of 20 sows were selected from 36 and 33 sows, from two farrowing batches one week apart. Due to an expansion of farm B in 2009, relatively few parity-1 sows were present and all gilts from the farrowing week batch were selected for follow up. Subsequently 76 selected sows were randomly allocated to pens in the farrowing room. Finally 871 piglets were sampled.

For analysis sow parity was divided into two groups, based on the observed median; parity 1 and 2 (referred to as 'low') and parity 3 and higher, referred to as