Actinobacillus pleuropneumoniae

Observational study on risk factors for infection of piglets with *Actinobacillus pleuropneumoniae* on a conventional pig farm

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1. Abstract

Actinobacillus pleuropneumoniae (APP) causes great economic losses in pig husbandry world-wide, due to pleuropneumonia. Direct contact is believed to be the main source of infection, but transmission via the air is demonstrated to be possible as well. This observational study was set up to gather information about the distribution of APP among sows, suckling piglets and weaned piglets on a conventional pig farm and to gain understanding in risk factors for infection with APP in the farrowing and weaner unit. Forty sows were tested three weeks pre-partum for the amount of antibodies for ApxI, ApxII, ApxIII and OMP and the amount of APP DNA present on the tonsils and their offspring was tested 3,5 weeks (all 40 litters) and 9,5 weeks (32 litters) post-partum for the presence of APP DNA on the tonsils. All sows were tested positive for antibodies and for the presence of APP DNA. At 3,5 weeks of age, 23% of the piglets, scattered over 55% of the litters, tested positive for the presence of APP DNA. No association was found between the chance of infection of a litter and the parity of the sow or the amount of present APP DNA or antibodies in the sow. Litters that were older at the moment of testing, had a higher chance of being infected than the younger litters. At 9,5 weeks of age, 42% of the piglets, scattered over 75% of the litters, tested positive for the presence of APP DNA. The infection chance was associated with the amount of antibodies for ApxI, but not with the amount of antibodies for ApxII, ApxIII and OMP. In the weaner unit, the presence of infected siblings was associated with the increase of infected piglets per pen, but the presence of infected piglets in the neighboring pens had no association with the infection of litters. This suggests that direct contact with infected piglets is the most important source of infection for piglets in the weaner unit and that entire litters may remain uninfected when direct contact with infected piglets is avoided.

Keywords: Actinobacillus pleuropneumoniae; observational study; transmission

2. Introduction

Actinobacillus pleuropneumoniae (APP) is a small, gram-negative, encapsulated rod-shaped bacterium that can cause pleuropneumonia in pigs. It is found world-wide and causes great economic losses in pig husbandry because of mortality, treatment costs and growth retardation[1-3].

Direct transmission is believed to be the main source of infection for pigs, rather than infection through environmental exposure[1]. Still Torremorell et al. (1997) and Jobert et al. (2000) demonstrated that diseased pigs can cause airborne transmission of the bacterium[2, 4].

To reduce the economic losses in pig farms, it is important to keep the prevalence of APP on farms as low as possible. Piglets can already become infected in the farrowing unit[3], where the sow is believed to be the main source of infection for the piglets[5]. Because direct contact with infected pigs is thought to be the main source of infection for pigs after weaning, it is likely that when infected pigs are present in the pen, pigs have a bigger chance of getting infected than when no infected pigs are present. Therefore, it is desirable to keep entire litters free from infection in the farrowing unit. It is, however, unknown what sow-bound or environment-bound factors are associated with the chance of infection of a litter in the farrowing unit. If risk factors for infection in the farrowing unit are found, it might be helpful in the development of measures to control infection with APP on farms.

The first goal of this study is to gather information about the ratio of infected and not infected sows on a pig farm and about the ratio of infected and not infected piglets in both the farrowing and the weaner unit and the distribution of infected piglets over pens. Another goal is to gain understanding in risk factors for infection with APP in the farrowing and the weaner unit of a pig farm. For this purpose, an observational study on a conventional pig farm has been performed. Sows were examined pre-partum on the amount of both APP DNA on the tonsils and antibodies against APP in serum and the sow's offspring was examined in the farrowing unit and in the weaner unit on the amount of present APP DNA on the tonsils. In the farrowing unit, associations between the chance of infection of a litter and sow-bound factors, like parity, concentration of antibodies and the amount of APP DNA in the sow's tonsils, were examined. In the weaner unit, associations between the infection chance and environment-bound factors, like the presence of infected piglets in the same pen and the presence of infected piglets in neighboring pens, were examined.

3. Theoretic background of *Actinobacillus pleuropneumoniae*

APP can be divided in 2 biotypes. Biotype I is nicotinamide adenine dinucleotide (NAD) dependent, while biotype II can synthetize NAD in the presence of a specific pyridine nucleotide or its precursors. Biotype I can be divided into 13 serotypes and biotype II is divided in 2 serotypes, based on the polysaccharide antigens on the surface[6, 7]. The association between biotypes and serotypes is not exclusive, since some biotype II strains belonging to serotypes that are normally found in biotype I strains, and opposite, have been found[1]. All virulent APP strains express 1 or 2 of the pore-forming exotoxins ApxI, ApxII or ApxIII. These toxins can cause lysis of several host cells, including alveolar epithelial cells, endothelial cells, erythrocytes and macrophages[7]. ApxI is strongly hemolytic and cytotoxic, ApxII is a little hemolytic and moderately cytotoxic and ApxIII is not hemolytic but strongly cytotoxic[8]. All serotypes can cause disease, but the virulence depends on the combination of produced Apx toxins, where the most virulent strains produce both ApxI and ApxII toxin[6]. Another toxin, ApxIV, is essential for full virulence and is expressed in all APP strains[7]. Factors that also determine the virulence of the bacterium include capsular polysaccharides, permeability factor and outer membrane proteins[9].

APP is believed to be unable to survive for long time in the environment, but when covered by organic matter it can survive for days or even weeks. In clean water, the bacterium is able to survive for 30 days at 4°C[1]. The bacterium is, also in the presence of organic matter, sensitive for several disinfectants, including Chloramine-T, hydrogen peroxide, glutaraldehyde and mercurochrome[10].

APP has developed several mechanisms to survive in the host. The mucociliary function is important in protecting the lower respiratory tract from infection. When the mucociliary function is suppressed, the bacterial multiplication exceeds the clearance rate and a substantial amount of bacteria can accumulate and increase the severity of disease[6]. In healthy animals, macrophages are the main phagocytes present in the lower respiratory tract, but the number of polymorphonuclear leukocytes (PMN's) increases rapidly following infection. PMN's can kill APP effectively after phagocytosis, whereas the bacterium can survive in macrophages for over 90 minutes, when the release of Apx toxins causes lysis of the macrophage[1, 6]. The capsular polysaccharide and/or LPS of APP cause resistance to complement-mediated opsonophagocytosis and to the bactericidal effects of serum[6]. In the lower respiratory tract, limited amounts of essential nutrients are available for the growth of bacteria. APP has several ways to obviate this problem, including inducing lysis of several cells, from which nutrients release in the environment[7]. The bacterium can survive for a long time in necrotic, and therefor low in oxygen, lung tissue. It produces several enzymes that enable anaerobic respiration of the bacterium[7].

Although the bacterium can be found in the upper respiratory tract, it doesn't bind well to the cilia or epithelial cells of the trachea or bronchi, but prefers the cilia of the terminal bronchioles and the epithelial cells of the alveoli. The aerosol particles that are produced by sneezing are small enough to penetrate the lower respiratory tract immediately, making it unnecessary to bind to the upper respiratory tract. How APP can spread to lung tissue in pigs that carry the bacterium in the tonsils is not known[6, 7]. The bacterium enters the lower respiratory tract mostly by inhalation as an aerosol. It colonizes by binding to the host cells, mucus and proteins, where it multiplies and produces substances that cause severe damage at these sites. Adhesion to host cells is possibly mediated by type 4 fimbriae and/or lipopolysaccharides, while the production of biofilms probably contributes to colonization[7].

Disease can occur in pigs of all ages, but is mostly seen in fatteners. Pigs of 12 weeks of age seem to be the most susceptible[7]. Clinical signs of the disease can occur in the peracute, acute or chronic form. In the peracute form, one or more pigs in the same or different pens become suddenly ill, with body temperatures rising up to 41°C, tachycardia, anorexia and apathy. The animals don't show evident respiratory signs, cardiac failure develops and the animal becomes cyanotic. In the last phase of the disease, the animals suffer from severe dyspnea and shortly before death a lot of

bloody, foamy discharge from pulmonary edema is seen from mouth or nose. Pigs die within 24-36 hours after development of clinical signs. In the acute form, many pigs in the same or different pens are affected. The animals suffer from anorexia, refuse to drink and body temperature may rise to 40.5-41°C. They have respiratory problems with dyspnea and coughing and cardiac failure may result in congestion of the extremities. Affected animals can die, become chronically infected or recover restless within the same infected group. The chronic form develops when the acute signs have disappeared. There is no or little fever, an intermittent cough and a reduced appetite, which contributes to growth retardation[1, 11].

 Isolation of APP can be achieved from the tonsils, lungs, nasal cavity and middle ear cavity of pigs[6]. At 4 weeks of age, APP can be detected in samples of the tonsils, but the presence in lung tissue is seen from 12-16 weeks of age[7]. Detection of APP from clinically healthy animals is complex. The tonsils are colonized with several other NAD-dependent bacteria, which may interfere with culture and the identification of APP[1]. Different PCR's are described that have higher specificity and sensitivity than the standard isolation method for the detection of APP[12].

4. Materials and methods

4.1. Animals and housing
Two groups of twenty sows were selected in a conventional pig farm in the south of the Netherlands with a history of APP and where 15% pleuritis is regularly found at slaughter, but with no recent history of clinical problems due to APP. The sows in each batch were selected of all sows based on expected parturition date and with an approximately even distribution of parities between the batches. The expected parturition date of the second batch was one week later than that of the first batch.

4.1.1. Sows
The sows were housed in a conventional group housing system in which the sows can freely move for most of the day. All higher parity sows of a batch were housed in the same room, but the gilts were housed apart from the sows. Three weeks before expected parturition date, tonsillar samples and blood samples were taken from all sows and gilts included in the experiment. Samples were marked by the ear tag number of the sow.

4.1.2. Farrowing system, hygiene and cross fostering
The farrowing rooms contain twenty pens. All sows of one batch were housed together in one room, where they were randomly assigned to the pens. Several hygiene and cross fostering measures were taken to obtain independent observations per pen. Piglets could be removed from all litters if the farmer found it necessary. However, the removed piglets could only be moved to three litters (further on: 'mixed litters') per room or to a litter in another room and were thereby excluded for follow-up. When the farmer shifted from one pen to another, he always changed gloves and overshoes. For the standard treatments (iron injection, tail docking and coccidiosis prevention) needles were changed between every pen. Together with the standard treatments, the piglets were ear tagged. Piglets were individually treated with antibiotics only when observed with disease by the farmer, except for the mixed litters where all piglets received penicillin with the standard treatment. At 3 days before weaning, at the age of approximately 3.5 weeks, tonsillar samples were taken from all piglets, marked by the ear tag number of the piglet.

4.1.3. Weaner unit
The weaner rooms consist of sixteen pens per room, so the three mixed litters and one randomly selected litter were transferred to a different room and removed from the study. The sixteen litters per batch that stayed in the experiment were litter-wise transferred to the weaner room where they were randomly assigned to the pens. Direct contact between piglets in different litters was minimized by the partition between the pens, as showed in figure 1. At approximately 9.5 weeks of age, tonsillar samples were taken from all piglets, marked by the ear tag number of the piglet. When the number of infected piglets in the neighboring pens was calculated with, only piglets that were already infected in the farrowing unit in the pens in the weaner unit directly next to the pen of interest were counted.

4.2. Samples and sample handling
Blood samples from sows were taken from the jugular vein and after 15 m centrifugation at 1500 g, serum was sent to MSD Animal Health for ELISA-testing on the amount of antibodies against ApxI, ApxII, ApxIII[13] and an outer membrane protein (OMP). The results are the $\log_2$ titers.

Tonsillar samples were obtained by brushing the surface of each tonsil for 5 seconds with a sterile toothbrush. The toothbrush was immersed in 10 mL 0.9% NaCl (saline) solution for 15 minutes. After mixing for 10 seconds, 1 mL of the fluid was stored at -20°C before DNA isolation and testing in duplicate by real-time quantitative PCR for the *ApxIV A* gene as described by Tobias et al. (2012)[14]. Genomic copies of PCR analysis of 5 were reckoned to be the lower limit, genomic copies of

PCR analysis <5 were considered to be negative. Because the sample was diluted 200 times before testing by PCR, the mean genomic copies as obtained by the PCR was multiplied by 200 to calculate the genomic copies of the sample (GC). Because of the lower limit of the PCR, the minimum possible GC was 1.0×10^3. The GC's were converted to the $\log_{10}$, before calculations were performed. Test results were identified and linked by the ear tag numbers.

FIGURE 1: Overview of a weaner room.

4.3. *Statistical analysis*

When data were divided into two groups, the median was taken as separation point and data equal to the median were assigned to the highest group. There was one exception: when parity was divided into two groups, the lower (first and second) parities were separated from the higher (third to ninth) parities.

In the farrowing unit, associations were evaluated between the chance of infection of litters and the batch, the age of the piglets at the moment of sampling, the parity, GC in the sows and amount of antibodies in the sows. In the weaner unit, associations were evaluated between the increase of infected piglets per litter or the chance of infection of a litter and the presence of infected siblings, the number of infected piglets in neighboring pens and the amount of antibodies in the sows. Litters were called infected, when at least one of the piglets of a litter was infected. Litters that were already entirely infected in the farrowing unit were excluded from tests that involved the increase of infected piglets per litter.

For all count data, association was tested with a chi-square test. When association was demonstrated, odds ratios (OR) were calculated. The correlations between results of different antibody tests were tested by Spearman's rank correlation coefficient, as were the correlations between parity and different antibodies.

OR and its 95% confidence intervals (95%CI) were calculated using MedCalc Software 12.3.0[15]. Other statistical analyses were performed using IBM SPSS 20.0 for Windows.

5. Results

5.1. Sows

The parity of the sows was distributed from parity 1 to parity 9, as displayed in table 1. There was no difference in lower (first and second) and higher (third to ninth) parity between batches (P=0.50).

All sows had positive test results in PCR for the presence of APP. The GC varied from 1.46 x 10^3 to 9.86 x 10^6. All sows had positive test result for antibodies for ApxI (with a minimum value of 10.4 and the maximum value of >14 in 5 sows), for ApxIII (with a minimum value of 10.4 and the maximum value of >14 in 7 sows) and for OMP (with a minimum value of 10.6 and the maximum value of >14 in 4 sows). 38 sows had a positive test result for ApxII with a minimum value of 13.1 and with the maximum value of >14 in 20 sows. Samples of the other 2 sows had an inconclusive test result and were excluded for tests that involved the ApxII titers. The correlation between the amount of antibodies in different tests showed a correlation of ρ=0.48 for ApxI and ApxII (P=0.002) and ρ=0,82 for ApxIII and OMP (P=0.00), but there was no correlation between the other antibody titers.

There was no correlation between the parity and both ApxI and ApxII (resp. P=0.21 and P=0,40), but a negative correlation was found between ApxIII and parity (ρ=-0.43, P=0.00) and between OMP and parity (ρ=-0.36, P=0.01).

TABLE 1: Distribution of individual parities over the batches.

	Batch 1	Batch 2
Parity 1	6	2
Parity 2	2	4
Parity 3	1	3
Parity 4	4	2
Parity 5	2	2
Parity 6	5	5
Parity 7	0	1
Parity 8	0	0
Parity 9	0	1
Total	**20**	**20**

5.2. Farrowing unit

479 piglets were tested three days before weaning at the age of 22 to 30 days. In total, 111 piglets (23%) tested positive for APP, scattered over 22 litters (55%) (further on: 'infected litters'), with a maximum GC of 1.86 x 10^7. An overview of the infected piglets per pen in each batch is displayed in figure 2. The number of infected piglets and infected litters per batch is displayed in table 2 and 3, respectively. There was no difference in number of litters per age group between the two batches, as displayed in table 4 (P=0.31).

In batch 1, 13 litters were infected while in batch 2 only 9 litters were infected, but there was no significant difference in the ratio of infected and not infected litters between batches (P=0.20). The number of infected piglets in batch 1 was significantly higher than in batch 2 (P=0.00).

4 of the 13 litters in the youngest age group (22-24 days old) were infected, while 16 of the 27 older litters (25-30 days old) tested positive, as displayed in table 5. With a P-value of 0.03 and an odds ratio of 4.5 (95% CI: 1.1-18.7), the ratio of infected and not infected litters was significantly higher in the older litters than in the younger litters.

The distribution of infected and not infected litters over parities is displayed in figure 3. There was no difference between lower (first and second) and higher (third to ninth) parity in the ratio of infected and not infected litters (P=0.84).

The GC of the sow was not associated with the ratio of infected and not infected litters (P=0.52), as displayed in table 6. None of the antibody titers had an association with the ratio of infected and not infected litters.

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FIGURE 2: Number of infected and uninfected piglets per pen in the farrowing unit.

2A: Batch 1

Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8	Pen 9	Pen 10
11+	3+	3+	0+	13+	0+	0+	4+	0+	7+
2-	6-	9-	12-	0-	12-	12-	7-	13-	5-

Pen 20	Pen 19	Pen 18	Pen 17	Pen 16	Pen 15	Pen 14	Pen 13	Pen 12	Pen 11
2+	6+	1+	0+	6+	0+	4+	3+	0+	1+
10-	6-	10-	12-	7-	14-	7-	8-	12-	9-

2B: Batch 2

Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8	Pen 9	Pen 10
0+	0+	0+	4+	1+	1+	0+	8+	0+	12+
10-	12-	12-	9-	12-	10-	12-	5-	12-	0-

Pen 20	Pen 19	Pen 18	Pen 17	Pen 16	Pen 15	Pen 14	Pen 13	Pen 12	Pen 11
8+	0+	1+	6+	0+	0+	0+	0+	0+	1+
5-	12-	11-	5-	13-	11-	14-	13-	11-	11-

White cells: litters with at least one infected piglet.
Green cells: litters without infected piglets.

TABLE 2: Infected and not infected piglets per batch in the farrowing unit.

	Batch 1	Batch 2	Total
Not infected piglets	168	200	**368**
Infected piglets	70	41	**111**
Total	**238**	**241**	**479**

TABLE 3: Infected and not infected litters per batch in the farrowing unit.

	Batch 1	Batch 2	Total
Not infected litters	7	11	**18**
Infected litters	13	9	**22**
Total	**20**	**20**	**40**

TABLE 4: Number of litters per age group per batch.

	Batch 1	Batch 2	Total
22-24 days old	5	8	**13**
25-30 days old	15	12	**27**
Total	**20**	**20**	**40**

FIGURE 3: Number of infected and not infected litters per parity.
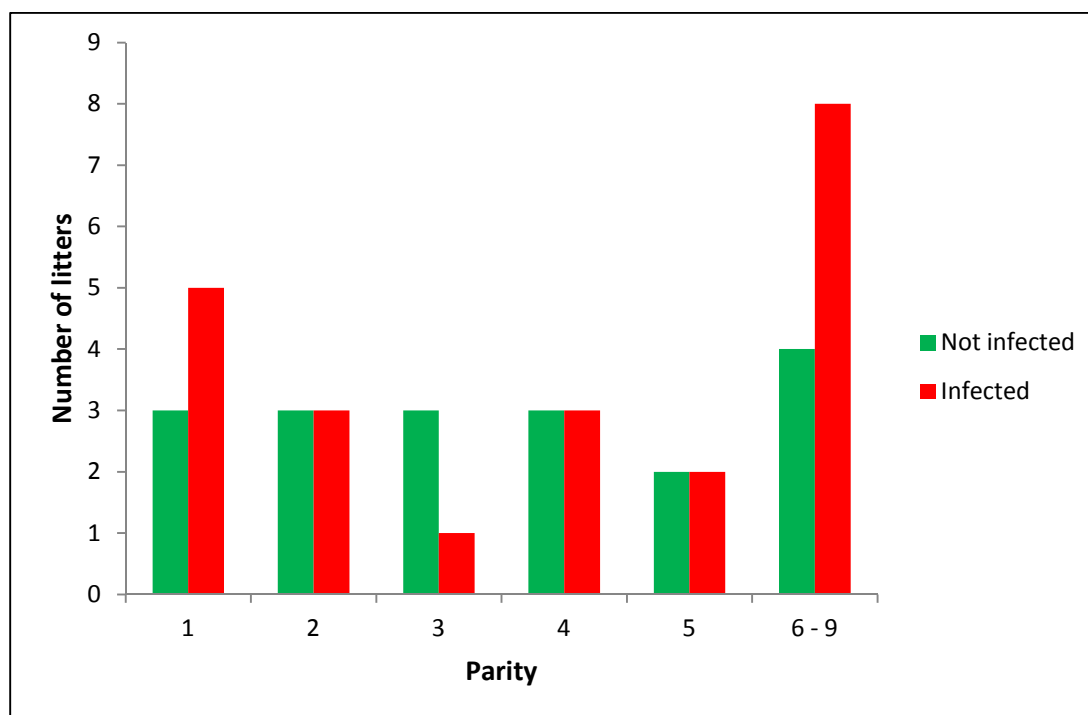
TABLE 5: Number of infected and not infected litters per age group.

	22-24 days old	25-30 days old	Total
Not infected litters	9	9	**18**
Infected litters	4	18	**22**
Total	**13**	**27**	40

TABLE 6: Number of infected and not infected litters per amount of GC in the sow.

	Low GC in sow	High GC in sow	Total
Not infected litters	8	10	**18**
Infected litters	12	10	**22**
Total	**20**	**20**	40

5.3. Weaner unit

From the 368 piglets that were tested at the age of approximately 9.5 weeks, 153 piglets (42%) scattered over 24 litters (75%) had a positive test result, with a maximum GC of 5.29 x 10^6 (see table 7 and 8). The distribution of the infected piglets over the pens is displayed in figure 4. In the farrowing system, 86 of these piglets scattered over 17 litters had a positive test result, which means that 7 of the 15 litters that weren't infected in the farrowing system got infected in the weaning unit. There was no difference in the ratio of infected and not infected litters between the batches (P=1.00), but there were still more infected piglets in batch 1 in proportion to batch 2 (P<0.01).

From the 15 litters where both infected and not infected siblings were present at weaning, 13 litters had an increase in the number of infected piglets, whereas only 7 of the 15 litters where no infected siblings were present at weaning had an increase of infected piglets (see table 9). The

presence of infected siblings was significantly associated with the increase of infected piglets in a litter (P=0.02 and OR=7.4 with 95%CI: 1.2 – 45.0).

From the 15 litters that weren't infected in the farrowing unit, 7 litters became infected in the weaner unit, from which only 2 had a high number of infected piglets in the neighboring pens (see table 10). There was too little data to get a significant test result, but there seemed to be no association between the number of infected neighboring piglets and the chance of infection in litters that were not infected in the farrowing unit. From the 14 litters with a high number of infected neighboring piglets, 8 litters had an increase in the number of infected piglets, while from the 16 litters that had a low number of infected neighboring piglets, 12 litters had an increase in the number of infected piglets (see table 11). There was no association between the number of infected neighboring piglets and the increase of infected piglets in a litter (P=0.30).

7 of the 15 litters from which the sow had a high titer of ApxI antibodies showed no increase in the number of infected piglets, while 13 of the 15 litters from sows with a low titer of ApxI antibodies showed increase in the number of infected piglets, as is displayed in table 12. With OR = 7.4 (95%CI: 1.2 – 45.0) and a P-value of 0.02, there was a significant difference in the increase of infected piglets in the weaner unit between low and high ApxI antibody titers. There was no correlation between the increase of infected piglets per litter in the weaner unit and the ApxII, ApxIII and OMP antibody titers in the sow.

FIGURE 4: Number of infected and uninfected piglets per pen in the weaner unit.

White cells: litters with at least one infected piglet.
Green cells: litters without infected piglets.

TABLE 7: The number of infected and not infected piglets per batch in the weaner unit.

	Batch 1	Batch 2	Total
Not infected piglets	89	125	**214**
Infected piglets	93	61	**154**
Total	**182**	**186**	**368**

TABLE 8: The number of infected and not infected litters per batch in the weaner unit.

	Batch 1	Batch 2	Total
Not infected litters	4	4	**8**
Infected litters	12	12	**24**
Total	**16**	**16**	**32**

TABLE 9: Increase in the number of infected piglets per pen when infected siblings were present or absent in the same pen in the weaner unit.

	No increase	Increase	Total
No infected siblings present	8	7	**15**
Infected siblings present	2	13	**15**
Total	**10**	**20**	**30**

TABLE 10: The number of infected and not infected litters in the weaner unit, that were not infected in the farrowing unit, with a low (0-4) or high (5-19) number of infected neighboring piglets.

	Not infected litter	Infected litter	Total
0-4 infected neighboring piglets	3	5	**8**
5-19 infected neighboring piglets	5	2	**7**
Total	**8**	**7**	**15**

TABLE 11: Increase of infected piglets per pen with a low (0-4) or high (5-19) number of infected neighboring piglets in the weaner unit. Only pens that were not completely infected in the farrowing unit.

	No increase	Increase	Total
0-4 infected neighboring piglets	4	12	**16**
5-19 infected neighboring piglets	6	8	**14**
Total	**10**	**20**	**30**

TABLE 12: Increase in the number of infected piglets per pen in the weaner unit from sows with a low or high concentration of antibodies against ApxI.

	No increase	Increase	Total
Low ApxI titer	2	13	**15**
High ApxI titer	8	7	**15**
Total	**10**	**20**	**30**

6. Discussion

In the farrowing unit, no association was found between the chance of infection of a litter with APP and the parity or the GC of the sow. Older litters were more often infected than younger litters. None of the antibody concentrations in the sow was associated with the chance of infection of a litter in the farrowing unit. In the weaner unit, high antibody concentrations for ApxI in the sow reduced the chance of infection of litters, while the antibody concentrations for ApxII, ApxIII and OMP had no association with the chance of infection. The chance of infection in the weaner unit was higher when infected piglets were present in the pen than when no infected piglets were present. The presence of infected neighboring piglets was not associated with the chance of infection.

In the farrowing unit, 18 of the 40 litters were not infected, which means that no transmission had occurred from the sow to the piglets, but also no transmission had occurred from infected litters to these litters. This suggests that the cross fostering and hygiene measures that were taken in the farrowing unit, paid off. In the weaner unit, 8 of the 32 litters were still not infected, so no transmission had occurred from infected litters to these litters, and the presence of infected piglets in the neighboring pens was found to have no association with the increase in the number of infected piglets per litter. This might suggest that airborne transmission hadn't played a big part in this experiment. In literature, however, can be found that transmission of APP can happen via direct contact or via airborne transmission over distances of 1 to 5 meter[2, 4]. This raises the suspicion that under field-like conditions where animals are naturally infected and show no disease related to APP, like in this experiment, airborne transmission plays not that big a part compared to animals which are experimentally inoculated with the bacterium and which do show clinical signs due to APP. This means that the results of this experiment cannot be extrapolated to animals that are diseased.

The age of piglets at the moment of sampling was found to be associated to the chance of infection of litters in the farrowing unit. For this test the group was divided into two parts, based on the median of ages of 25 days. Hereby was chosen to allocate the litters with the age equal to the median, a group of 11 litters, to the group with the oldest ages. This was done because in all other cases where the group was divided into two, based on the median, the cases equal to the median were allocated to the highest group. If, however, all litters with the age of 25 days would have been allocated to the group with the youngest litters, no association would have been found between the chance of infection of litters and the age of the piglets. This supposes that the association between the age and the infection chance of litters is not distinct.

The piglets in the farrowing unit were sampled three days before weaning. In the interpretation of the results in the weaner unit, the results of the farrowing unit were taken as starting point for the weaner unit, with the assumption that no litters or piglets had been infected in the last three days in the farrowing unit. The chances are, however, not negligible that some piglets had been infected by the sow in the last days in the farrowing unit. This makes it difficult to explain the new infections in the weaner unit of 7 of the 15 litters that weren't infected in the farrowing unit. Were they infected via the air by neighboring piglets in the weaner unit, or were they still infected in the farrowing unit by the sow after all?

In this experiment, no relation was found between infection chance of litters and the serum level of antibodies in the sow in the farrowing unit and in the weaner unit, only a relatively high concentration of antibodies to ApxI seemed to have a negative association with the increase of infected piglets per pen. In literature, however, can be found that offspring from sows with high antibody titers for APP serotype 2 have higher concentrations of serum antibodies from birth to the age of 9 weeks compared to offspring of sows with low antibody titers[16]. This suggests that offspring of sows with high antibody titers are better protected for infection with APP in the first two months of their lives. Sjölund et al. (2011) included only sows with a relatively low or high antibody concentration, while in this experiment all sows were included. The inclusion of sows with antibody concentrations close to the median concentrations makes the difference between both groups less

distinct. The groups were based on the median concentrations on this farm and it is not known how high the concentrations on this farm are in comparison to other farms in the Netherlands. To determine whether high or low antibody concentrations have an association with the chance of infection of litters, only sows with high and low antibody concentrations should be included in the test. Therefore, the antibody concentrations of the sows should be compared with other farms and more sows should be tested.

Sows were sampled three weeks before parturition in this experiment. It is not clear how the levels of antibodies and the GC in the sow changed in the last three weeks pre-partum. Sjölund et al. (2011) found that the serum concentration of antibodies to APP serotype 2 remained at the initial level in sows with low levels of antibodies in the last month before parturition, but the level decreased in sows with high antibody levels[16].

Due to the analytical sensitivity of 1000 GC per sample it is not inconceivable that several samples are erroneously called negative. With the follow-up of the piglets, 8 piglets that were tested positive for the presence of APP in the farrowing unit, tested negative in the weaner unit. In the farrowing unit they had GC varying from 1.29×10^3 to 6.59×10^5, in the weaner unit 1 of the piglets had a GC<1000, the other 7 piglets had GC = 0. Because of the high GC of some of the piglets in the farrowing unit, it was thought to be unlikely that the tests in the farrowing unit were false positive. Some, but not all, of these piglets had received antibiotics, but even when antibiotics are used it is unlikely that the piglets completely eliminated the bacterium. It was therefore chosen to consider the test results of these 8 piglets from the weaner unit being false negative and so the piglets were considered to be still infected in the weaner unit. It is however debatable whether this is the right assumption and, if it is, how many more piglets were tested false negative.

Both in the farrowing unit (40 litters) and in the weaner unit (32 litters), the amount of data was relatively small. Therefore, the chance of coincidence and thus type II errors is quite big and it is difficult to explain whether the absence or presence of association between data is real or based on coincidence.

To gain more understanding in the transmission of APP in non-diseased animals and risk factors for infection, more research is necessary. It is advisable to repeat this experiment on different farms, so results can be compared between farms and more data is available for statistical testing. It is thereby desirable to include more animals per farm, so better interpretation of results within farms is possible and more selection of data is possible for specific statistical tests, without increasing the chance of coincidence in the test. It is also important to do more research into the differences in transmission of APP between non-diseased animals and diseased animals.

The goals of this study were gathering information about the ratio and distribution of infected and not infected animals and gaining understanding in risk factors for infection with APP in the farrowing and the weaner unit. In this farm, all sows were infected with APP and had antibodies against APP. In the farrowing unit, 55% of the litters was infected with APP and in the weaner unit, 75% of the litters was infected. No sow-bound factors were found to have an association with the chance of infection of litters in the farrowing unit. In the weaner unit, the presence of infected siblings was associated with the increase of infected piglets per pen, but the presence of infected piglets in the neighboring pens had no association with the infection of litters. This suggests that direct contact with infected piglets is the most important source of infection for piglets in the weaner unit and that entire litters may remain uninfected when direct contact with infected piglets is avoided.

7. References

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