Establishing the underlying causes of pleurisy to enable the development of effective prevention and treatment measures.

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Executive Summary

Introduction

Studies overseas have shown us that pleurisy does not only has an enormous impact on the production but also at slaughter. Studies linking the observed pleurisy at slaughter back to the farm have shown post-weaning mortality rates are around 3.3% higher for farms with a > 10% pleurisy at slaughter than farms that are not affected. Models for pleurisy have predicted that in increase of 1% pleurisy relates to a reduction of 1.55-2.5g/day average daily lifetime weight gain at batch level. At the abattoir, pleurisy slows down the line speed and leads to more waste and compromised carcass weights. In recent years, an increase of pleurisy at the abattoir has been observed worldwide. This observed increase in pleurisy, which has been noted in abattoirs in Australia, and the fact that no knowledge of the bacterial and viral species associated with pleurisy at the abattoir existed in Australia has led to this study.

The study was undertaken at an abattoir in Queensland that had information about the pleurisy observed for different farms and contributed that information for the farms sampled. This situation allowed us to sample high and low pleurisy farms and determine which pathogens were found in pigs with pleurisy.

Method

A total of 46 batches of pigs representing 46 Queensland farms were sampled. Five lungs affected with pleurisy were sampled from most of these farms. At the abattoir the sex, weight and back fat measure of each pig sampled was taken and the average weight and back fat from the sampled batch was recorded. In the laboratory, all lungs were photographed, scored and samples taken from pleurisy affected areas and the underlying bacterial species cultured from these samples. All growth on the plates was scored to provide a crude estimate of the level of bacteria found in the lungs. Lungs were also tested for *Mycoplasma hyopneuomiae* by testing the trachea and the apical lobes. The bronchial lymph node was tested for PCV2. All respiratory associated bacterial species were identified via PCR or sequencing. For some of the species further analysis was undertaken, such as serotyping.

One species present on most of the farms was used to test for the antimicrobial sensitivity.

Results

The outcome was a large list of respiratory pathogens found with the most prevalent being *M. hyopneumoniae* and *Streptococcus suis*, found on 34 and 38 farms, respectively. PCV2 was found in high concentration in samples from 29 farms. The other pathogens found were *Pasteurella multocida* (24 farms), *Actinobacillus* species (29 farms), *Actinobacillus pleuropneumoniae* (7 farms), *M. hyorhinis* (4 farms), *M. flocculare* (9 farms), *Streptococcus porcinus* (1 farm), *S. minor* (1 farm), *Haemophilus parasuis* (1 farm) and Bisgaard Taxon 10 (1 farm). Most farms had more than one species of bacteria.

When taking into consideration the level of bacteria found in the affected lungs, which was determined by the plate score, most bacteria were there in only low numbers. However, some species, such as *S. suis*, *P. multocida* and *A. pleuropneumoniae*, were in high numbers, indicating that they were potentially the causative agents of the pleurisy.

Estimation of the univariable odds ratio showed the crude risk of a positive result for *M. hyopneumoniae* was higher for pigs coming from batches with a batch pleurisy score greater than ten percent compared to those from batches with pleurisy scores less than ten percent.

Discussion

It is probably not surprising that no single infectious cause for all instances of pleurisy was found in this study, as chronic pleurisy is supposed to be attributed to a variety of pathogens. A variety of pathogens were found with only four being regarded as primary important respiratory pathogens - *M. hyopneumoniae, A. pleuropneumoniae, H. parasuis* and PCV2.

Of these primary pathogens, only *M. hyopneumoniae* and PCV2 were found on a large percent of farms with *M. hyopneumoniae* associated with the farm pleurisy status. These two pathogens are regarded as pathogens that act synergistically, which means that these two low pathogenic agents in combination with another low pathogenic agent can cause severe respiratory disease. All other bacterial species found are typically regarded as secondary pathogens, ie pathogens with low virulence, another point that makes the observation of high prevalence of *M. hyopneumoniae* and PCV2 an important observation.

The surprising results were the high prevalence of *S. suis* in the lung of pigs at slaughter with this species being the most common species detected. All *S. suis* found were type 1 and no type 2 was found, which is the most common cause of disease in humans. It is known that most pigs are carriers of *S. suis*, and that it can cause issues throughout the production cycle, but most outbreaks occur between three and 12 weeks. Therefore, finding it in large numbers in the lungs of apparently healthy slaughter age animals is surprising.

The recommendation from this research are that a revisit of the protocols in place for the control of *M. hyopneumoniae* and PCV2 is necessary. A further look at the levels of *S. suis* on farms might also be advisable.

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1. Introduction

Surveillance of animal disease at abattoirs is an intrinsic part of the quality control performed by the abattoir. It is also used by veterinarians to obtain a picture of the health status of a particular farms. The two predominant respiratory disease condition found during routine abattoir surveillance are lung consolidation most likely cause by enzootic pneumonia seen as confluent consolidation affecting the cranioventral regions of the lungs and pleurisy (Eze et al, 2015; Fabley et al, 2012). Pleurisy (pleuritis), which is a finding not characteristic for a particular disease denoting inflammation of the pleura seen as fibrous or fibrinous adhesions on the lung or between the lung and the chest wall, which has a considerable impact on the cost of pig production due to reduction in growth performance and feed efficiency with 8 to 12 days delay to slaughter (Christensen et al 1999; Merialdi et al 2011). As well, chronic pleurisy has been associated with increased time for slaughter due to the extra time required for trimming the carcass and slower production line speed. It also brings with it more waste and compromised carcass weights and grades (Jaeger et al. 2012). In England the cost of pleurisy has been estimated to be as high as £ (Sterling) 2.30 for every single pig slaughtered (http://www.pigprogress.net/Health-Diseases/2013/2/Pleurisy).

Pleurisy has been reported to be on the increase, with a rise from 16 to 20.8% of individual affected pigs from 2000 to 2009 in Belgium, from 14 to 24% from 1987 to 1998 in Denmark, and from 12 to 22.5% individual affected pigs from 1990 to 2004 in the Netherlands (Jaeger et al 2012). Fablet et al (2012a) reported 3.8 to 62% of pigs as being affected by pleurisy depending on the country and the lung lesion scoring system used. The increase of pleurisy has been noted in slaughterhouses in Australia and recently at Swickers, Kingaroy (personal communication).

Respiratory disease can be split up into acute and chronic. The acute form if not fatal leads to speedy recovery, while the chronic form is presentative of a long standing problem, which leads to a prolonged or permanent damage from which the pig never recovers. Chronic disease in a population is more complex involving several agents, among them bacteria and viruses, which might act synergistically. Of the viruses, only Porcine Circovirus type 2 is relevant to Australia. The bacteria associated with chronic respiratory disease are *Mycoplasma hyopneumoniae, Mycoplasma hyorhinis, Haemophilus parasuis, Actinobacillus pleuropneumoniae, Actinobacillus suis, Streptococcus suis, Pasteurella multocida, Bordetella bronchiseptica* and *Arcanobacter pyogenes* (www.nadis.org.uk). The frequency of the bacterial strains present appears to differ from country to country. A survey of pleuritis and

pulmonary lesions in pigs at an abattoir in Italy revealed that 25.1% of the lungs had dorsocaudal pleuritis with a high percentage of these lungs affected by dorsocaudal pleuritis being associated with *A. pleuropneumoniae* according to blood samples and herd health history (Merialdi et al. 2011). In Spain, pleuritis and cranio-ventral pulmonary consolidation lesions were recorded in 26.8% and 55.7% of pigs at slaughter, respectively, with a total of 82% of the farms sending the pigs to slaughter testing positive to *A. pleuropneumoniae* and *M. hyopneumoniae* (Fraile et al 2010). In Canada, a study sampled the tonsils from 180 normal carcasses and from 215 carcasses diverted to the hold rail, the latter representing carcasses with identified concerns or visible defects that require a closer inspection by veterinary officials (O'Sullivan et al 2011). The most common bacterial species found were *S. suis* (53.7%), *A. pyogenes* (29.9%), *P. multocida* (27.3%) and *S. porcinus* (19.5%) (O'Sullivan et al 2011). A study in France revealed 69.3% of lungs at slaughter had pneumonia and 15% had pleuritis and *M. hyopneumoniae, P. multocida, A. pleuropneumoniae, S. suis* and *H. parasuis* were detected in the lungs (69.3, 36.9, 20.7, 6.4 and 0.99%, respectively) (Fablet et al 2012b). In a Danish study looking at pigs at slaughter (pigs with and without pleurisy), a strong association was found between chronic pleurisy and *M. hyopneumoniae, A. pleuropneumoniae, P. multocida* and *H. parasuis* (Enoe et al 2002). A Norwegian study that examined 855 pig lungs at slaughter recovered *P. multocida, A. pleuropneumoniae* and *Streptococcus* spp. from pneumonic lesions (54, 11 and 14%, respectively) (Høie et al 1991). In the Netherlands, the increase in pleuritis in slaughter pigs over the last twenty years prompted a study that revealed 45% pleuritis, 14% pleuropneumonia and 38% catarrhal pneumonia. While *A. pleuropneumoniae* and *P. multocida* were found, no single infectious cause of pleuritis was found, rather a variety of infectious agents together with environmental factors were considered the cause of pleuritis (Jirawattanapong et al 2011). A review on polymicrobial respiratory disease in Iowa stated that, despite widespread vaccination, *M. hyopneumoniae* is still a major concern for pig producers (Opriessnig et al 2011). The veterinary Diagnostic Laboratory at Iowa State University noted that in 2010 *P. multocida* and *M. hyopneumoniae* were the main bacterial pathogens recovered from respiratory disease cases (Opriessnig et al 2011).

A study to determine the influence of PCV2 on pleuritis and lung lesions found that the detection of IgM antibodies against PCV2 in piglets 20 – 22 weeks of age correlated with a reduced rate of pleuritis or lung lesions at slaughter (Wellenberg et al 2010). In 58% of herds with a high level of lung lesions, the level of PCV2 DNA was above 10,000 PCV2 DNA copies/mg lung tissue (which they defined as a high level) compared to 29% in herds with

low level of lung lesions. Eleven out of the 19 affected lungs from herds with high level of lung lesions had a high PCV2 DNA load in combination with one or more other lung pathogens, while this combination was only found in five out 17 affected lungs from herds with low levels of lung lesions (Wellenberg et al 2010). The observation of PCV2 underpinning pleurisy was also noted in the large English Pleurisy study by BPEX (Tucker et al 2000).

Summary

In summary, there seems to be no doubt that there is an overall increase of pleurisy at slaughter in a number of countries, and possible including Australia. The bacterial species associated with pleurisy and their prevalence is dependent on the country where the research was undertaken. The bacterial species seen in several countries were *M. hyopneumoniae, P. multocida* and *A. pleuropneumoniae*, followed by *S. suis* and *H. parasuis* in at least two studies. PCV2 infection is increasingly being recognised as an important factor contributing to pleurisy and lung disease. The knowledge of bacterial/virus species contributing to this increase in pleurisy, the antimicrobial sensitivity status of the contributing bacterial species is needed not only for the development of effective treatment on the farm, but also for effective preventative measures to be implemented. Combining lung scoring, bacterial load, knowledge of bacterial/viral species, antibiotic sensitivity patterns, abattoir data for farm and farm management, farm vaccination programs and farm health status, will help to define addition risk factors besides the management risk factors already defined by Colin Cargill (Health Welfare Production and Housing Optimisation Program – DAS 1616 PRDC 2001).

2. Methodology

Lung at Abattoir

Lungs were collected from an abattoir in Queensland. The abattoir (via the work of Kingsley Krefford) had monitored the pleurisy of batches (pigs send in from a farm to slaughter on a particular day) of pigs from farms over the years and had rated the farms as low, intermediate and high pleurisy. Monitored pigs were given an abattoir pleurisy score of one if one side of the lung attached to the parietal pleura and a score of two if both sides of the lungs were attached. A farm was described as a low pleurisy farm if consistently less than 10% of pigs

in monitored batches had a score of either one or two. A farm was described as a high pleurisy farm if consistently more than 20% of pigs in each batch had a score of either one or two. A farm that either had between 10 and 20%, or was varying between low and > 10% or high and < 20% was considered variable.

One batch of pigs per farm was sampled with a target of five lungs per batch. Lungs, this included the left and right side of the lung with trachea and lymph nodes attached (referred to as lung throughout), were collected from pigs with a score of either 1 or 2 using a systematic sampling process with a sampling interval of 1, 2, 3, 4 or 5; the interval depended on the batch size and the pleurisy category of the farm. From most farms, five lungs were collected. However, from the low pleurisy farms collecting five lungs was not always possible and lower numbers of lungs were collected from some farms.

Each lung was placed into a zip lock freezer bag labelled with the farm brand (which was later on coded to avoid identification of the farm) and lung number and put on ice. The laboratory was three hours away from the laboratory and lung sampled in the morning were held in the cold room at the abattoir before the travel back to the laboratory. The ice was topped up in the eskies before the drive back. At the laboratory, the lungs were held overnight on ice before processing the following day.

For each pig that had a lung sampled, the weight, sex and back fat was recorded. The average weight (calculated from the HSC (hot standards carcass weight) of each pig) and back fat of the pigs in the total batch were recorded.

The lungs were collected every second week over a period of nine months (July 2014 to March 2015). A total of 210 lungs were collected from 46 farms associated with 24 owners and 7 pork supply chains. Each farm in this trial was coded with a four digital number. The first number presented the pork supply chain with a number being from 01 to 11. The next digit presented the farm owner and the fourth number presents the farm of that owner. Some owners owned up to eight farms, run by different managers.

At the first, blood was collected from the axillary vessels for PCV testing from pigs of two farms, but not enough blood could be collected and the line speed was interrupted. Therefore, the tracheobronchial lymph nodes were collected from pigs from all subsequent farms and used to test for PCV2.

At the laboratory the lungs were photographed and then scored. The cranio-ventral pneumonia scoring described by Pointon et al. (1999) was used. Each lung lobe was scored for the percent of lesions and consolidation with the highest total score awarded being 55. This indicated the proportion of consolidated lung tissue in the following sites: a maximum

score of 10 was assigned to each of the apical and cardiac lobes, 5 for each intermediate lobe and 5 for the caudal lobes.

Cranio-ventral pneumonia lesions were classified as either acute or chronic and the absence or presence of pleuropneumonia lesions associated with *Actinobacillus pleuropneumoniae* were recorded. A lung pleurisy score was also given ranging from 0 to 3 with lung pleurisy score 1 being small amount fibrin on lung surface, lung pleurisy score 2 being fibrin over extensive area of lung, lung pleurisy score 3 being lung lobes stuck together or lung stuck to rib cage

Culture

Plates were labelled with the farm code, lung number and sample number. Both sides of the lung were sampled and cultured separately. The samples taken where from sites of adhesion. If no adhesion were observed on one side, then that side was sampled at the equivalent position as the side with the adhesions. Four cultures plates were used per sample: BA/SN agar plate (consisting of blood agar based medium, BBLTM Blood Agar Base (BD), supplemented with 0.0025% of NADH, 0.0005% of thiamine HCl, 1% of heat inactivated horse serum and 5% of oleic acid bovine albumin complex (consisting of 4.75% bovine serum albumin in normal saline (containing 0.06% oleic acid and 5% 0.05 N NaOH)), blood agar plate, *Streptococcus* selective media plate and McConkey agar plate. All plates were incubated for a minimum of 48 hours under aerobic conditions at 37ºC. An additional swab was taken from each sample site for DNA extraction directly from the swab. Additionally, a 500 µl of fluid from the zip lock bag that had held the lung overnight was taken for DNA analysis.

Two samples were collected from each pig for *Mycoplasma* spp analysis. A swab inserted deep into the trachea was taken and processed by PCR. For the other sample, one apical lobe was cut off and stored in a plastic bag with 3 ml of added phosphate buffered saline (PBS) overnight at 4ºC. The next day 500 µl of fluid was taken off and used for PCR analysis.

Culture analysis

The BA/SN plates, which were the first ones to be inoculates, were scored according to the following scoring system:

0 = no growth

1 = colonies growing only in directly swabbed area

2 = colonies growing directly in swabbed area and in first streak only

3 = colonies growing in directly swabbed area and in first and second streaks

4 = colonies growing in directly swabbed area and in first, second and third streaks

Colonies of suspect respiratory pathogens (such as members of the *Pasteurellaceae* family) were picked and re-plated for identification. From the BA/SN agar plates nicotinamide adenine dinucleotide (NAD) dependent species were recovered, while from the blood agar plate *P. multocida* and other bacteria were collected. *Streptococcus*-like bacteria were collected from the *Streptococcus* selective agar plate. The McConkey agar plate was used to help in the selection of respiratory pathogens compared to environmental bacteria and to help in the isolation and identification of *E. coli*.

Potentially different bacterial species were selected according to their colony appearance and plated onto fresh plates. The DNA was extracted after overnight incubation and the isolate was stored at -70ºC.

DNA extraction

DNA extraction from swab

The cotton end of the swab was placed into a 1.5 ml tube. To this swab tip, 500 ul of PBS was added and the tube vortexed for 15 seconds. After being allowed to stand for 10 mins, the tube was vortexed again. (Depending on work load, the second vortexing was performed after overnight storage). The swab was removed from the tube by releasing as much liquid as possible from the swab. The remaining suspension was centrifuged for 5 mins at 13000 x g. The DNA was then extracted with the Qiagen DNeasy blood & Tissue Kit (cat no 69504). The first step was to add 180 µl Buffer ATL and then 20 µl proteinase K was added and the mixture vortexed thoroughly. The method was then followed according to instructions given in the manufacturer's instructions.

DNA extraction from lung fluid

To a 1.5 ml tube 500 µl of lung tissue fluid was added and spun at 1,000 g for 3min. The supernatant was transferred to another tube and spun for 5 min at 13,000 rpm. After discarding of the supernatant, 180 µl of Buffer ATL was added and the DNA was extracted according to the manufacturer's instructions of the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany).

DNA extraction for cultured bacteria

The DNA of gram-positive bacteria was extracted with the Generation Capture Column kit of Qiagen (Qiagen, Maryland, USA). The DNA of gram-negative bacteria species was extracted via the boiling and cooling process according to the PCR protocol (see identification paragraph below).

Identification

The suspect *A. pleuropneumoniae* were identified and serotyped with a multiplex *A. pleuropneumoniae* PCR for serovars 1, 5, 7 and 15 (Turni et al., 2014). This multiplex PCR both identifies at the species level (i.e. confirms as *A. pleuropneumoniae*) and identifies if the isolate is serovar 1, 5, 7 or 15.

Suspect *Streptococcus suis* isolates were identified with a PCR based on the glutamate dehydrogenase gene (Okwumabua et al., 2003), as well as a PCR based on the 23S rDNA, which identifies down to species level as well as type 1 or 2 (Kawata et al 2004). Other *Streptococcus* spp isolated from the selective medium were tested with another PCRs. The PCR of Kawata et al (2004), which can identify eight streptococcal species relevant to animal infections (*Streptococcus agalactiae, S. bovis, S. canis, S. dysgalactiae, S. equi, S. porcinus, S. suis* and *S. uberis*), was used.

Mycoplasmas were identified in the lung tissue fluid and from the tracheal swab with a multiplex PCR for *M. hyopneumoniae, M. hyorhinis* and *M. flocculare* according to the protocol of Stakenborg et al (2006). *P. multocida* was identified with a species specific PCR (Townsend at al 1998) and then the LPS genotype established by PCR (Harper et al, 2015). All other potential pathogens were identified by16S rDNA sequencing (Blackall et al. 2003).

Antibiotic sensitivity

Sixty-six *S. suis* isolates from 38 farms were tested by the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013) for antimicrobial susceptibility to nine antimicrobials (ampicillin, ceftiorfur, erythromycin, florfenicol, penicillin, cotrimoxazole (trimethoprim/sulfamethoxazole), tetracycline, tilmicosin and tulathromycin). The interpretation guidelines were taken from *Streptococcus* spp from humans in the case of ampicillin, erythromycin, penicillin, cotrimoxazole and tetracycline and for *S. suis* for ceftiorfur and florfenicol– all were as provided by the Clinical and Laboratory Standards Institute (CLSI, 2015).

PCV2

One gram of lymph node was used to extract DNA with the QIAGEN tissue kit (Qiagen, Maryland, USA). The real time PCR was run according to the protocol of Olvera et al (2004). Unfortunately, while shifting the lymph nodes to another laboratory one box of the samples, representing 5 farms, went missing and therefore only lymph nodes from 39 farms were tested.

Data management and statistical analyses

Sample size was dictated by logistics with the recognition that the study would have limited power to detect an association between identification of a pathogen and batch/farm pleurisy categories.

Data for two samples from each lung were combined for each bacteria species meaning if one sample was positive the lung was considered positive for the bacteria species. The results for the apical and tracheal mycoplasma results were also combined and a positive result for one sampling site meant the lung was positive. If the results for one test were missing and the tested location was negative then no overall result was recorded. A total pathogen count was calculated based on the number of the following pathogens to which the pig was positive: *M. hyopneumoniae*, *M. hyorhinis*, *A. pleuropneumoniae*, *S. suis*, *P, multocida* L3, *P. multocida* L6, *P. multocida* NT and PCV2. Dichotomous variables were then created for whether the pathogen count was (i) 0/≥1, (ii) <2/≥2, (iii) <3/≥3.

Dichotomous plate score variables were also created by first determining the greater of the plate scores for the two samples for each pig, then categorizing that as (i) <2/≥2, (ii) <3/≥3.

The farms were recorded according to the abattoir's rating of high, variable and low pleurisy farm (farm with overall lower score than 10% over observed period were classed as low, farms with >20% were classed as high and farms that varied between low and above or high and below or where in between the low and the high classification were classified as variable).

In addition to the farm pleurisy categories based on retrospective data, a batch pleurisy categories were defined whereby batches were categorized as ≤10% and >10% according to the percentage of pigs in the batch that had an abattoir pleurisy score of either one or two pleurisy.

Continuous variables were summarized by mean, standard deviation, median, minimum and maximum. Categorical variables were summarized by frequency distributions at the farm, batch or pig level, as appropriate.

To assess the extent of clustering of each pathogen of interest by farm intra-class correlation coefficients were estimated. This was done by fitting separate null logistic regression models for each pathogen of interest with farm as a random effect. To test the null hypotheses that there is no association between the farm/batch pleurisy category and likelihood of a positive result for each agent a series of multi-level univariable logistic regression models were fitted with the result for each infectious agent as the outcome variable and either batch pleurisy category or farm pleurisy category as the exposure variable and farm as the random effect. This was only been done if at least five pigs had a positive result for the infectious agent

An odds ratio of one indicates no association. An odds ratio of greater than one indicates that the odds of a positive test result are greater in that category than the reference category – e.g. an odds ratio of two means that the odds of a positive result are twice as high for pigs with that category of the variable than pigs with the reference category of the variable. An odds ratio of less than one indicates that the odds of a positive result are lower in that category compared to the reference category. The low batch pleurisy category and the low farm pleurisy category were set as reference categories, so that if one expects an odds ratios greater than one if the infectious agent is more likely to be found in pigs from higher pleurisy batches or variable/high Swickers farm scores.

3. Outcomes

A total 46 batches from 46 farms were sampled with an average batch pleurisy score of 17.3%, average weight per pig of 80 kg, average back fat of 11.1 mm and a mean of 150 pigs per batch. Of the total 210 lungs sampled more lungs were collected from batches with a pleurisy score above ten percent (Table 1). Mean weight of pigs were similar across pleurisy categories. The cranio-ventral pneumonia (CVP) mean score was 5.9 for lungs from batches with ≤ 10% pleurisy compared to 12.0 for lungs from batches with >10%.

Table 1. Summary statistics for continuous variables at farm/batch and pig levels. Pig level variables are summarised for all pigs by batch and Swickers pleurisy categories. CVP – cranio-ventral pneumonia. HSC – hot standard carcass (weight of carcass on hooks).

Variable	Number	Mean	Median	S.D.	Minimum	Maximum
Farm/Batch						
Batch pleurisy score	46	17.3	15.0	15.0	0.8	63.0
HSC weight average (kg)	46	80.0	82.3	12.1	10.1	92.6
Back fat average (mm)	46	11.1	11.0	1.6	8.7	18.0
Number of pigs in batch	46	150	160	68.7	40	325
Pig						
Weight						
All pigs	210	80.7	81.6	8.7	52.6	101.7
Batch ≤ 10%	75	80.3	81.5	7.9	59.2	97.0
Batch > 10%	135	80.9	81.7	9.1	52.6	101.7
Swickers pleurisy category						
Swickers Low	62	79.6	82.3	10.3	52.6	100.5
Swickers Var	93	81.3	81.3	7.8	60.0	101.7
Swickers High	55	81.0	80.2	8.2	65.8	97.6
CVP score						
All pigs	210	9.8	6	11.2	0	51
Batch ≤ 10%	75	5.9	2	8.0	0	38
Batch > 10%	135	12.0	9	12.1	0	51
Swickers pleurisy category						
Swickers Low	62	8.2	1	8.2	0	38
Swickers Var	93	12.3	9	11.8	0	51
Swickers High	55	10.8	6	11.6	0	41

More batches were being sampled for the above 10% pleurisy (Table 2) category and from most farms five lungs were collected. A total of 64.3% of the lungs sampled came from a batch with a higher than ten pleurisy percentage (Table 3).

Table 2. Frequency distribution of pleurisy categories by farms/batches and number of pigs sampled per farm

Variable/Category	Total	Percentage
Batch score		
≤ 10%	19	41.3
> 10%	27	58.7
Swickers pleurisy category		
Swickers Low	16	34.8
Swickers Var	19	41.3
Swickers High	11	23.9
No. pigs sampled		
1	1	2.2
2	2	4.4
3	3	6.5
4	4	8.7
5	36	78.3

Table 3. Frequency distribution of pleurisy categories by pigs

Variable/Category	Total	Percentage
Batch score		
≤ 10%	75	35.7
> 10%	135	64.3
Swickers pleurisy category		
Swickers Low	62	29.5
Swickers Var	93	44.3
Swickers High	55	26.2

Looking at the distribution of lung scores the batches with >10% pleurisy seem to have a shift towards the higher scores (Figure 1). Most lungs (95.7%) had a lung pleurisy score of 3 for the lungs and 50.5% were of chronic infection with most of these coming from the batches with pleurisy > 10% (70.8%). At the abattoir most lungs (66.2%) had an abattoir pleurisy score of 1 (one side of the lung attached) (Table 4).

Figure 1. Histograms of cranio-ventral pneumonia scores by batch pleurisy category and overall

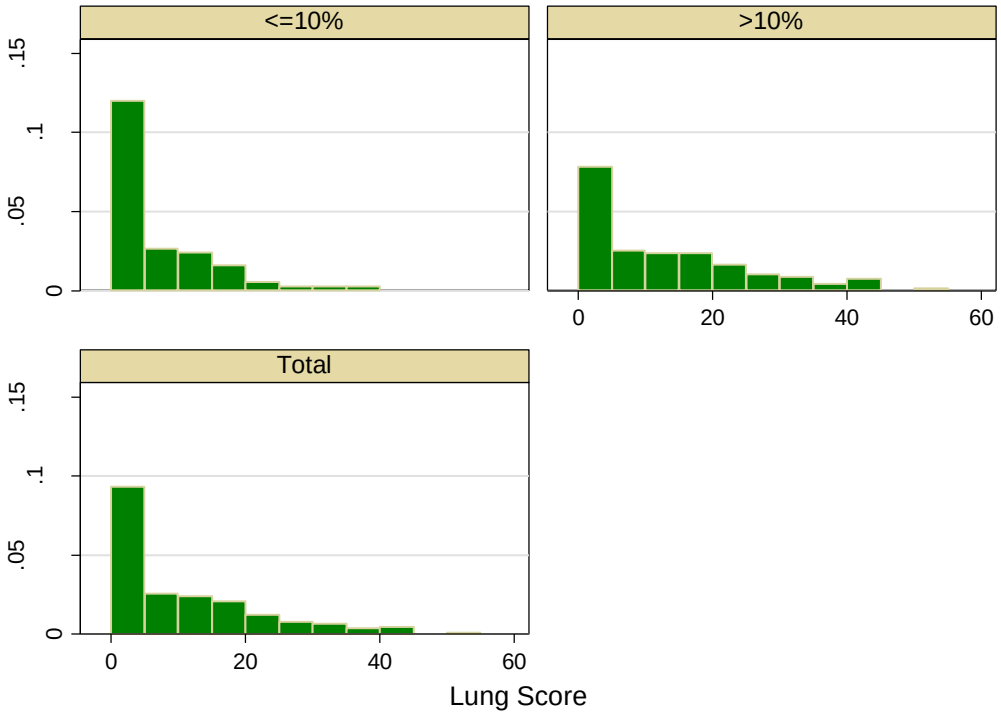


Table 4. Frequency distribution of categorical variables relating to gross findings at the pig level for all pigs and by batch and Swickers pleurisy category. CVP – cranio-ventral pneumonia. A/C – acute/chronic. APP – *Actinobacillus pleuropneumoniae*

Variable/ Category	Total Number (%)[1]	Batch pleurisy category		Swickers pleurisy category		
		Batch ≤ 10% No. (%)[2]	Batch > 10% No. (%)[2]	Low No. (%)[2]	Variable No. (%)[2]	High No. (%)[2]
Gender						
Female	123 (58.6)	49 (39.8)	74 (60.2)	40 (32.5)	57 (46.3)	26 (21.1)
Male	87 (41.43)	26 (29.9)	61 (70.1)	22 (25.3)	36 (41.4)	29 (33.3)
Abattoir pleurisy score						
1	139 (66.2)	50 (36.0)	89 (64.0)	42 (30.2)	59 (42.4)	38 (27.3)
2	71 (33.8)	25 (35.2)	46 (64.8)	20 (28.2)	34 (47.9)	17 (23.9)
Lung pleurisy score						
1	0 (0)	0	0	0	0	0
2	9 (4.3)	3 (33.3)	6 (66.7)	4 (44.4)	2 (22.2)	3 (33.3)
3	201 (95.7)	72 (35.8)	129 (64.2)	58 (28.9)	91 (45.3)	52 (25.9)
Stage of CVP						
None	51 (24.3)	26 (51.0)	25 (49.0)	27 (52.9)	12 (23.5)	12 (23.5)
Acute	27 (12.9)	12 (44.4)	15 (55.6)	13 (48.1)	5 (18.5)	9 (33.3)
A/C	26 (12.4)	6 (23.1)	20 (76.9)	5 (19.2)	8 (30.8)	13 (50.0)
Chronic	106 (50.5)	31 (29.2)	75 (70.8)	17 (16.0)	68 (64.2)	21 (19.8)
APP lesions						
No	202 (96.2)	70 (34.7)	132 (65.3)	60 (29.7)	89 (44.1)	53 (26.2)
Yes	8 (3.8)	5 (62.5)	3 (37.5)	2 (25.0)	4 (50.0)	2 (25.0)

[1]Column percentage e.g. 58.6% of all pigs were female

[2]Row percentage e.g. 39.8% of female pigs came from batches where the batch pleurisy score was ≤ 10%

Table 5 shows the frequency distribution of pathogens and their degree of clustering by farm. *M. hyopneumoniae, P. multocida, S. suis,* and PCV2 were most commonly identified and strongly clustered by farm. *M. hyopneumoniae* and *S. suis* were present on 34 and 38 out of 46 farms, respectively. *P. multocida* was present on 24 farms out of 46 and PCV2 was present on 29 farms out of 39 tested. *A. pleuropneumoniae, M. flocculare, A. porcinus* also cluster by farm, however, they were not that commonly found. *E. coli* is also clustered by farm, but it is not regarded as a respiratory pathogen and would point to contamination rather than an infective agent.

Table 5. Frequency distribution of infectious agents (identified by culture or PCR) by pig and farm (all farms and farms with 5 pigs sampled). The intra-class correlation co-efficients (ICC) and associated p values assess the degree of clustering of each infectious agent by farm.

Infectious agent	No. +ve pigs	No pigs with results	% +ve	No. +ve farms	No. +ve farms with 5 samples	ICC	P value
M. hyopneumoniae	110	209	52.6	34	29	0.62	<0.001
M. hyorhinis	4	208	1.9	4	4	<0.01	0.499
M. flocculare	15	209	7.2	9	8	0.54	0.001
A. pleuropneumoniae	9	210	4.3	7	7	0.38	0.039
P. multocida[2]	45	210	21.4	24	20	0.23	0.012
P. multocida L3	24	210	11.4	14	13	0.42	0.001
P. multocida L6	20	210	9.5	15	12	0.19	0.118
P. multocida NT	4	210	1.9	3	3	0.54	0.075
S. suis	118	210	56.2	38	31	0.33	<0.001
S. porcinus	1	210	0.5	1	1	<0.01	0.499
S. minor	1	210	0.5	1	1	<0.01	0.499
A. porcinus	9	210	4.3	6	5	0.54	0.006
A. minor	12	210	5.7	10	9	0.14	0.288
A. porcitonsillarum	12	210	5.7	12	11	<0.01	1
E. coli	37	210	17.6	21	17	0.29	0.004
H. parasuis	1	210	0.5	1	1	<0.01	0.498
Bisgaard Taxon 10	1	210	0.5	1	1	<0.01	0.499
PCV2[1]	79	179	44.1	29	24	0.45	<0.001

[1]Pigs from only 39 farms were tested for PCV
[2]*P. multocida* results are presented as all *P. multocida* (ie *P. multocida*), only those *P. multocida* that were confirmed as LPS PCR type 3 (*P. multocida* L3), only those *P. multocida* that were confirmed as LPS PCR type 6 (*P. multocida* L6) and only those *P. multocida* that could not be assigned to a LPS PCR type (*P. multocida* NT).

When looking at the species that have been found per farm (Table 6) there seems to be no apparent association with any one species and high batch pleurisy score and the multitude of species does not seem to be associated with the batch pleurisy score either, however that is not taking in consideration multiple lungs per farm. Only two of the low pleurisy farms have no bacterial species, but one of them has a high viral load. *P. multocida* is seen on farms at all levels of pleurisy (from low to very high pleurisy percentage). *A. pleuropneumoniae* was seen only at pleurisy above 8%. Interestingly, a lot of *Actinobacillus* species are found on these farms with 29 farms having at least one species.

Table 6 Bacteria species and PCV2 virus found on each farm together with the batch pleurisy score of the batch sampled. PCV2 is given as the number of copies per gram of tissue for the lung with the highest concentration per farm.

Code for farm	Total pleurisy score (%) for batch sampled	Bacteria species found for each farm								PCV2 (log 10)
		App	PM	*S. suis*	*Actinobacillus* sp.	*M. hyopneumoniae*	*M. hyorhinis*	*M. flocculare*	*Others*	
0451	0.83									8.66
0831	1.26									not done
0881	1.49		LPS 6	Type 1		✓			*H. parasuis*	neg
1012	1.75			Type 1						8.86
0863	1.76			Type 1	*A. minor*					9.18
0884	1.79			Type 1	*A. porcitonsillarum*	✓				7.15
0817	1.85			Type 1				✓		8.96
0882	1.99		LPS 6	Type 1	*A. indolicus*	✓				neg
0414	3.48			Type 1	*A. porcitonsillarum*	✓		✓		7.51
0862	3.8			Type 1						neg
0461	4.52		LPS 6, LPS NT	Type 1	*A. porcitonsillarum, A. porcinus*	✓				7.26

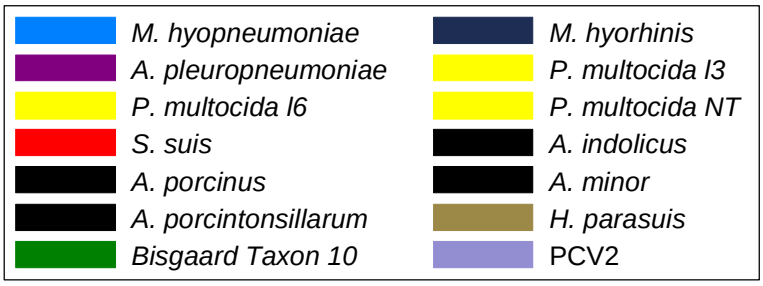
0616	5		LPS 3			✓			neg
0413	5.08		LPS 3		*A. minor*	✓			10.21
1055	5.88			Type 1		✓			8.46
1041	7.84		LPS 6	Type 1	*A. porcinus*	✓			not done
0822	7.94			Type 1	*A. porcinus, A. minor*	✓			not done
0814	8.73		LPS 3	Type 1		✓			8.09
0415	8.89	Serovar 15	LPS 6		*A. minor*			✓	not done
1021	9.3			Type 1		✓			9.70
0615	10.46			Type 1	*A. indolicus, A. porcitonsillarum*	✓	✓		neg
0462	11.93			Type 1	*A. porcinus*	✓			6.96
1031	13.66	Serovar 7		Type 1		✓			9.39
0443	14.52		LPS 6, LPS NT	Type 1	*A. porcitonsillarum*	✓			11.26
0871	15.48		LPS 6	Type 1	*A. indolicus, A. minor*	✓		✓	neg
0611	16.46		LPS 3	Type 1		✓			7.28

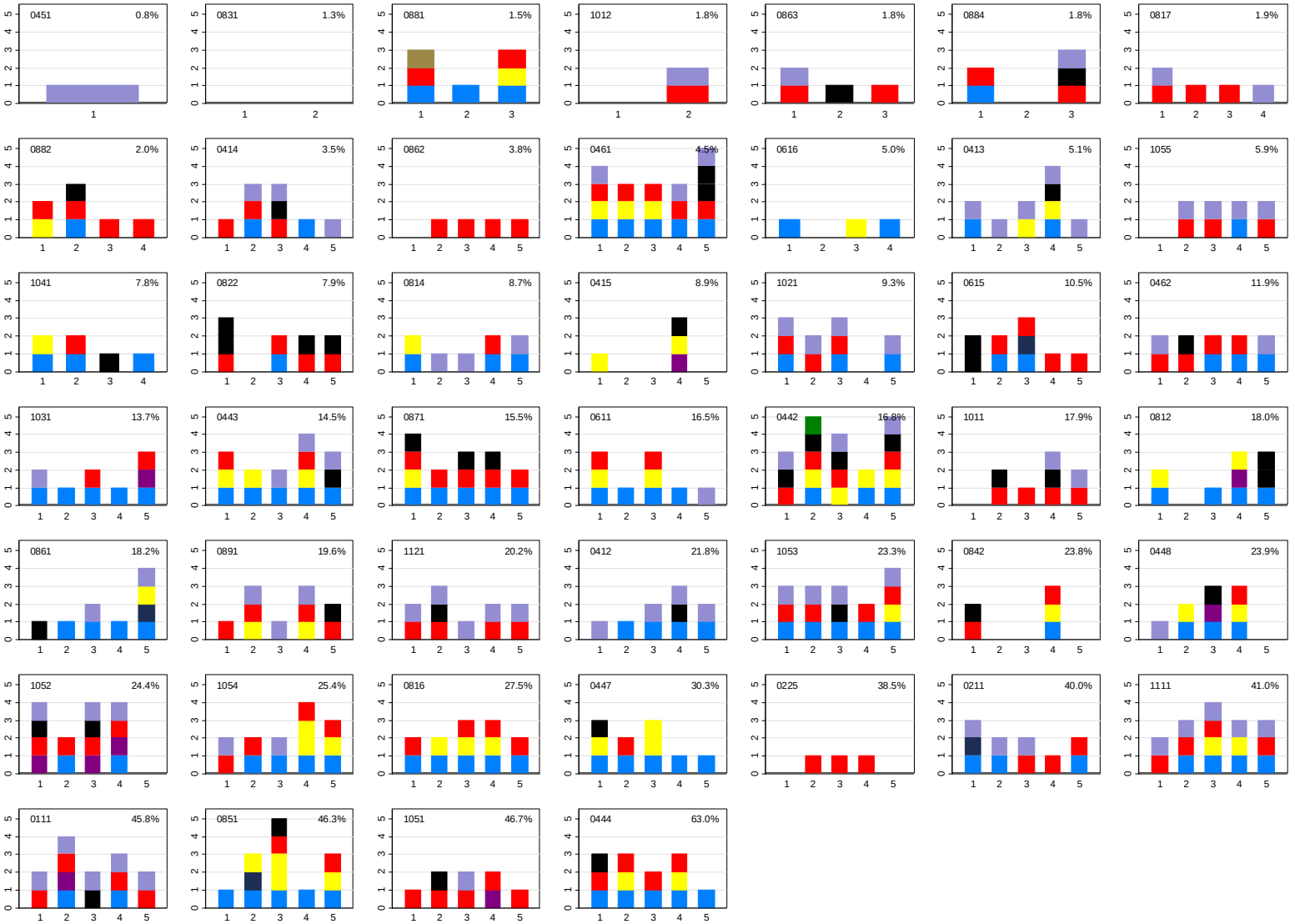
0442	16.82		LPS 3	Type 1	*A. porcitonsillarum, A. minor, A porcinus*	✓	✓	Bisgaard Taxon 10	9.87
1011	17.89			Type 1	*A. indolicus, A. minor*			*S. minor*	7.29
0812	18.04	Serovar 7	LPS 3 + LPS 6		*A. minor, A. porcitonsillarum*	✓	✓	*S. porcinus*	not done
0861	18.18		LPS 3		*A. indolicus*	✓	✓		8.57
891	19.58		LPS 3, LPS 6	Type 1	*A. indolicus*				8.38
1121	20.16			Type 1	*A. porcitonsillarum*		✓		9.06
0412	21.76				*A. porcitonsillarum*	✓			10.66
1053	23.31		LPS 6	Type 1	*A. indolicus*	✓			10.48
0842	23.81		LPS 3	Type 1	*A. indolicus*	✓			
0448	23.94	Serovar 15	LPS 3	Type 1	*A. minor*	✓			6.49
1052	24.36	Serovar 7		Type 1	*A. porcinus*	✓			8.16
1054	25.45		LPS 3,	Type 1		✓			7.99

			LPS 6						
0816	27.46		LPS 6	Type 1		✓		✓	neg
0447	30.26		LPS 3, LPS 6, LPS NT	Type 1	*A. porcitonsillarum*	✓			neg
0225	38.46			Type 1					not done
0211	40			Type 1		✓	✓		10.34
1111	41.01		LPS 3	Type 1		✓			10.68
0111	45.81	Serovar 7		Type 1	*A. porcitonsillarum*	✓			7.48
0851	46.3		LPS 3, LPS 6	Type 1	*A. porcitonsillarum*	✓	✓		neg
1051	46.73	Serovar 7		Type 1	*A. indolicus*				9.48
0444	62.96		LPS 6	Type 1	*A.minor*	✓			neg

[1]For all bacteria, no entry means that the agent was not detected and ✓ indicates that the specific agent was identified. For PCV2, some samples could not be tested and are recorded as Not Done.

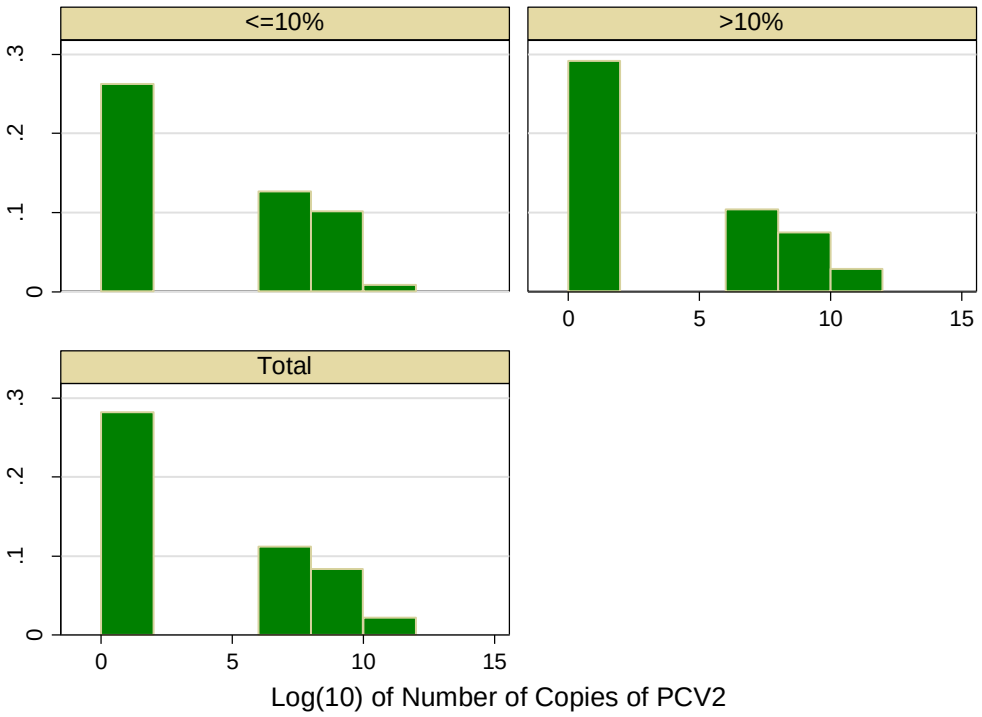
Figure 2 This figure shows all the species on the farms ordered by batch pleurisy score and representing each lung from each batch with all species. Each square represents one farm with 1 to five lungs. The figure in the right hand corner is the pleurisy score of the batch the lungs came from. The right hand corner figure is the farm code. The *Actinobacillus* species have been combined, as well as all the *P. multocida* of all serovars.




0451 0.8%
0831 1.3%
0881 1.5%
1012 1.8%
0863 1.8%
0884 1.8%
0817 1.9%
0882 2.0%
0414 3.5%
0862 3.8%
0461 4.5%
0616 5.0%
0413 5.1%
1055 5.9%
1041 7.8%
0822 7.9%
0814 8.7%
0415 8.9%
1021 9.3%
0615 10.5%
0462 11.9%
1031 13.7%
0443 14.5%
0871 15.5%
0611 16.5%
0442 16.8%
1011 17.9%
0812 18.0%
0861 18.2%
0891 19.6%
1121 20.2%
0412 21.8%
1053 23.3%
0842 23.8%
0448 23.9%
1052 24.4%
1054 25.4%
0816 27.5%
0447 30.3%
0225 38.5%
0211 40.0%
1111 41.0%
0111 45.8%
0851 46.3%
1051 46.7%
0444 63.0%

When looking at all the lungs per farm and looking at the species found in all lungs (Figure 2) no clear association can be seen either. There seems to be some farm where not all lungs harboured pathogens and some where all lungs from one farm harboured pathogens, even though not necessarily all the same pathogens. The distribution of PCV2 levels appears similar across batch pleurisy categories (Figure 3).

Figure 3. Histograms of log (10) of number or copies of PCV2 (assuming zero for those with a negative PCV2 result) by batch pleurisy category and overall



Most of the BA/SN plates had a score of 1 for their plate count, which means that the bacteria were only in the base streak and not in large number (Table 6). When looking at the plates with higher score (3 and 4) most of the high scores are caused by *S. suis* (Table 7). However, some of the high plate scores also had *P. multocida* and *A. pleuropneumoniae* on them. Some of the plates had environmental bacteria on them, as can be seen in the list of others/contaminants.

Table 6 Frequency distribution of plate counts for each sample and combined

Sample	No. pigs	%
Sample 1		
0	7	3.3
1	147	70.0
2	33	15.7
3	17	8.1
4	6	2.9
Sample 2		
0	5	2.4
1	136	64.8
2	46	21.9
3	20	9.5
4	3	1.4
Combined		
1	10	4.8
2	103	49.1
3	48	22.9
4	26	12.4
5	17	8.1
6	4	1.9
7	1	0.5
8	1	0.5

Table 7. Associated bacteria with high plate scores.

Farm code	Plate score	App serotype	PM serotype	S. suis	A. porcinus	A. minor	A. poritonsillarum	Others/Contaminants
0111	3	App 7		S. suis				
0211	3			S. suis				
0412	4							Streptococcus spp
0412	4						A. porsitonsillarum	Streptococcus spp
0414	3							Streptococcus spp
0415	4	App 15	PM L6			A. minor		
0415	3							E. coli, Anoxybacillus flavithermus
0444	3		PM L6	S. suis				
0444	3			S. suis				
0447	3		PM L3				A. porsitonsillarum	
0447	4		PM L6 + LNT					
0448	4		PM L3					
0461	4		PM LNT	S. suis				
0461	4			S. suis				
0461	3			S. suis				
0461	3							Streptococcus spp
0462	3			S. suis				
0462	3				A. porcinus			
0462	3			S. suis	A. porcinus			
0611	3		PM L3					
0615	3			S. suis				
0814	3		PM L3					
0816	3			S. suis				
0822	3			S. suis				
0842	3		PM L3					
0842	3							Streptococcus spp
0862	3			S. suis				
0862	3							Anoxybacillus kaynarcensis
0871	3		PM L6	S. suis				
0871	3			S. suis				
0871	4			S. suis				
0881	3							Streptococcus spp
0882	3		PM L6	S. suis				
0882	3			S. suis				
0884	3							Streptococcus spp
0891	3							Streptococcus spp
1021	3							?
1021	4							?
1031	3							?
1051	3			S. suis				
1052	3							?
1111	3							Streptococcus spp
1111	3			S. suis				
1121	3						A. porcitonsillarum	
1121	3			S. suis				

There was no association between the plate score and the pleurisy categories. However, when looking at eight pathogens, *M. hyopneumoniae, M. hyorhinis, A. pleuropneumoniae, P. multocida* L3, *P. multocida* L6, *P. multocida* LNT, *S. suis* and PCV2, there was evidence of an association between higher pathogen score (more pathogens present) and pleurisy categories (Table 8).

Crude risk of a pathogen score ≥ 2 was higher for pigs coming from batches with a pleurisy score of >10% compared to those from batches where the pleurisy was ≤ 10% (odds ratio (OR): 2.8, 95% confidence interval (CI): 1.2 – 6.9). Similarly crude risk of a pathogen score ≥3 was higher for pigs coming from farms in the variable and high pleurisy categories compared to the low pleurisy category (ORs: 4.3, 95% CI: 1.2 – 15.5 and 7.6, 95% CI 1.8 – 31.9, respectively). Similar but less precisely estimated trends were seen for a pathogen score ≥ 2 and the farm pleurisy category and a pathogen score ≥ 3 and the batch pleurisy category (Table 8).

Table 8. Number and percentage of pigs with a positive result for dichotomous plate score and pathogen count variables by batch and Swickers pleurisy categories. Estimated univariable odds ratios for the association between pleurisy categories and the occurrence of a positive result. Bolded p values are overall likelihood ratio test p values. Non-bolded p values are individual Wald test p values

Infectious agent Variable/category	No (%) pigs positive	Odds ratio (95% CI)	p value
Plate Score ≥2			
Batch pleurisy score			**0.763**
≤ 10%	34 (45.3)	Reference	
>10%	64 (47.4)	1.1 (0.5 - 2.3)	0.763
Swickers pleurisy category			**0.423**
Low	29 (46.8)	Reference	
Variable	39 (41.9)	0.8 (0.4 - 1.8)	0.600
High	30 (54.5)	1.4 (0.6 - 3.4)	0.447
Plate Score ≥3			
Batch pleurisy score			**0.793**
≤ 10%	15 (20.0)	Reference	
>10%	25 (18.5)	0.9 (0.4 - 1.9)	0.793
Swickers pleurisy category			**0.369**
Swickers Low	14 (22.6)	Reference	
Swickers Var	19 (20.4)	0.9 (0.4 - 1.9)	0.749
Swickers High	7 (12.7)	0.5 (0.2 - 1.3)	0.171
Pathogen score ≥1[1]			
Batch pleurisy score			**0.064**
≤ 10%	52 (88.1)	Reference	
>10%	115 (95.8)	3.1 (0.9 - 10.2)	0.063
Swickers pleurisy category			**0.406**
Swickers Low	45 (90.0)	Reference	
Swickers Var	83 (93.3)	1.5 (0.4 - 5.3)	0.497
Swickers High	39 (97.5)	4.3 (0.5 - 38.7)	0.189
Pathogen score ≥2[1]			
Batch pleurisy score			**0.021**
≤ 10%	30 (50.8)	Reference	
>10%	86 (71.7)	2.8 (1.2 - 6.9)	0.021
Swickers pleurisy category			**0.145**
Swickers Low	27 (54.0)	Reference	
Swickers Var	58 (65.2)	1.7 (0.7 - 4.6)	0.258
Swickers High	31 (77.5)	3.4 (1.0 - 11.9)	0.050
Pathogen score ≥3[1]			
Batch pleurisy score			**0.073**

≤ 10%	10 (16.9)	Reference	
>10%	37 (30.8)	2.8 (0.9 - 8.9)	0.073
Swickers pleurisy category			**0.020**
Swickers Low	5 (10.0)	Reference	
Swickers Var	26 (29.2)	4.3 (1.2 - 15.5)	0.025
Swickers High	16 (40.0)	7.6 (1.8 - 31.9)	0.006

[1]Results are for 179 pigs from 39 farms

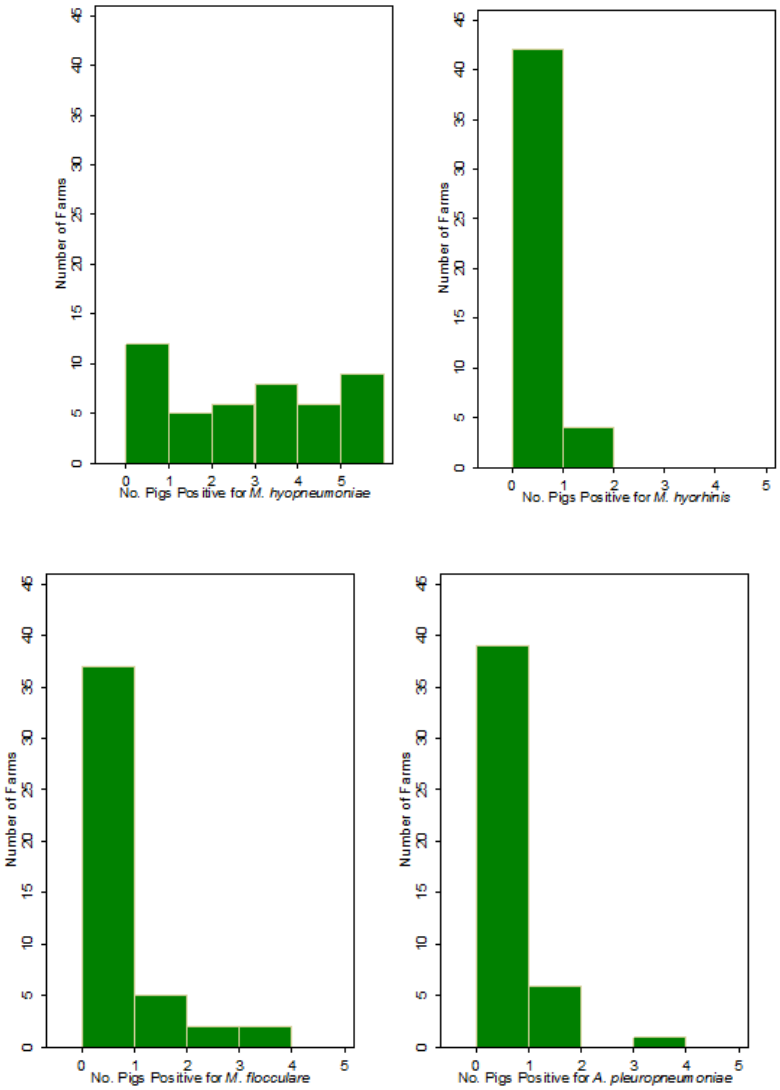
When looking at eight pathogens, *M. hyopneumoniae, M. hyorhinis, A. pleuropneumoniae, P. multocida 3, P. multocida 6, P. multocida NT, S. suis* and PCV2, most of the pigs (32.9%) harboured more than one of these respiratory pathogen, while 12 pigs (5.7%) harboured none of these pathogens (Table 9).

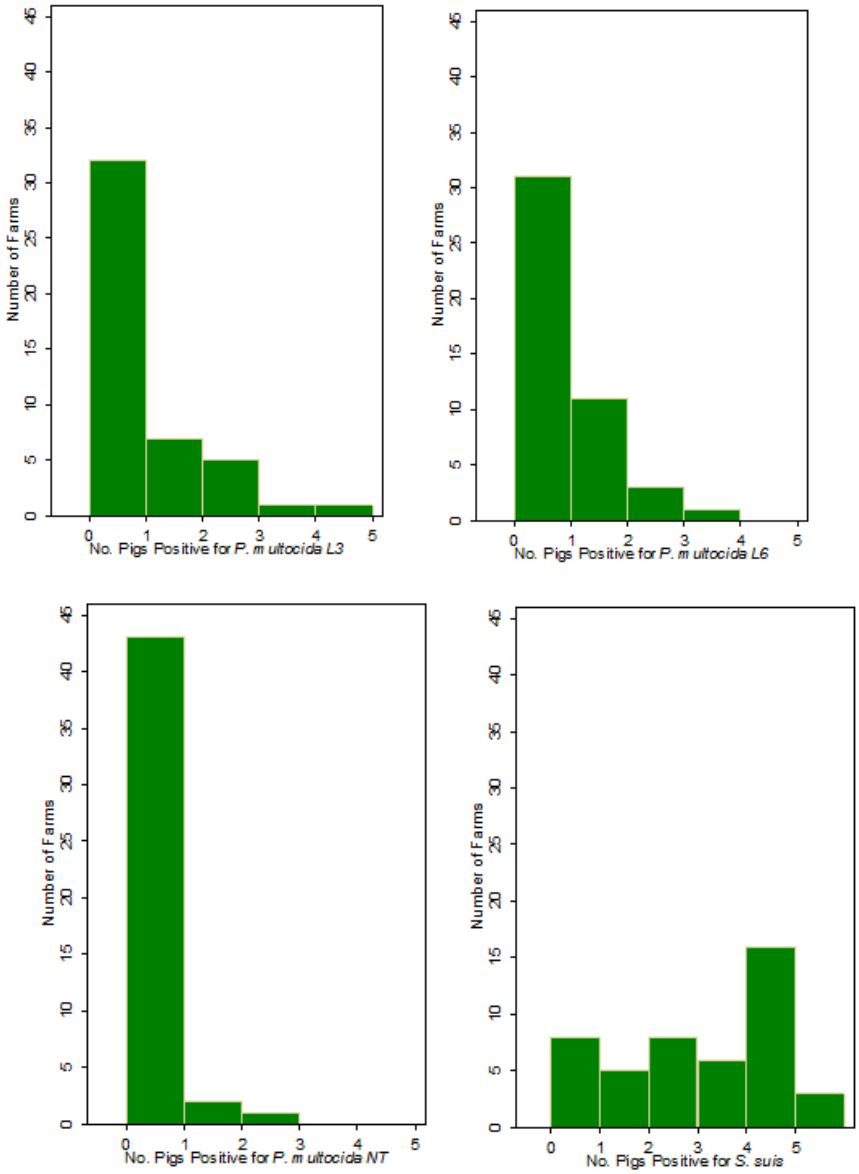
Table 9. Frequency distribution of eight pathogen (*M. hyopneumoniae, M. hyorhinis, A. pleuropneumoniae, P. multocida 3, P. multocida 6, P. multocida NT, S. suis* and PCV2) counts for each pig

Pathogen Count	No. pigs	% pigs
0	12	5.7
1	51	24.3
2	69	32.9
3	37	17.6
4	10	4.8
Missing	31	14.8

Of the *Mycoplasma* species *M. hyopneumoniae* was the most prevalent being present in 52.6% of the pigs sampled, present on 34 out of 46 farms and present on some farms in all pigs sampled (Figure 4). *S. suis* was also present in high numbers sampled from 56.2% of the lungs, present on 38 farms and on same farms it was present in all pigs sampled. *P. multocida* L3 was also retrieved from some farms in all pigs sampled.

Figure 4. Farm/batch level frequency distribution of the number of pigs testing positive to *Mycoplasma* species, *A. pleuropneumoniae*, *P. multocida* L3, *P. multocida* L6, *P. multocida* NT and *S. suis*.





Estimated univariable odds ratios for the association between pleurisy categories and the occurrence of a positive test result for each agent are shown in Table 10. Crude risk of a positive result for *M. hyopneumoniae* was higher for pigs coming from batches with a batch pleurisy score of >10% compared to those from batches where the pleurisy was ≤ 10% (odds ratio (OR): 6.5, 95% confidence interval (CI): 1.3 – 31.5). Similarly crude risk of a positive result for *M. hyopneumoniae* was higher for pigs coming from farms in the variable and high pleurisy categories compared to the low pleurisy category (ORs: 20.3, 95% CI: 3.6 – 114.0 and 11.1, 95%CI 1.6 – 76.4, respectively).

Table 10 Number and percentage of pigs with a positive result for each infectious agent by batch and Kingsley's pleurisy categories. Estimated univariable odds ratios for the association between pleurisy categories and the occurrence of a positive test result for each agent. Odds ratios were only estimated when there were at least five pigs testing positive. Bolded p values are overall likelihood ratio test p values. Non-bolded p values are individual Wald test p values

Infectious agent Variable/category	No (%) pigs positive	Odds ratio (95% CI)	p value
M. hyopneumoniae			
Batch pleurisy score			**0.020**
≤ 10%	27 (36.0)	Reference	
>10%	83 (61.9)	6.5 (1.3 - 31.5)	0.020
Swickers pleurisy category			**0.003**
Swickers Low	15 (24.2)	Reference	
Swickers Var	64 (68.8)	20.3 (3.6 - 114.0)	0.001
Swickers High	31 (57.4)	11.1 (1.6 - 76.4)	0.014
M. hyorhinis			
Batch pleurisy score			
≤ 10%	0 (0.0)	-	
>10%	4 (3.0)	-	
Swickers pleurisy category			
Swickers Low	0 (0.0)	-	
Swickers Var	2 (2.2)	-	
Swickers High	2 (3.8)	-	
M. flocculare			
Batch pleurisy score			**0.986**
≤ 10%	6 (8.0)	Reference	
>10%	9 (6.7)	1.0 (0.1 - 7.1)	0.986
Swickers pleurisy category			**0.092**
Swickers Low	10 (16.1)	Reference	
Swickers Var	4 (4.3)	0.2 (0.0 - 1.3)	0.097
Swickers High	1 (1.9)	0.1 (0.0 - 1.2)	0.067
A. pleuropneumoniae			
Batch pleurisy score			**0.180**
≤ 10%	1 (1.3)	Reference	
>10%	8 (5.9)	5.2 (0.5 - 58.6)	0.180
Swickers pleurisy category			**0.087**
Swickers Low	1 (1.6)	Reference	
Swickers Var	2 (2.2)	1.4 (0.1 - 18.7)	0.811
Swickers High	6 (10.9)	8.4 (0.7 - 94.9)	0.085
P. multocida			
Batch pleurisy score			**0.216**
≤ 10%	12 (16.0)	Reference	
>10%	33 (24.4)	1.9 (0.7 - 5.0)	0.216
Swickers pleurisy category			**0.443**
Swickers Low	10 (16.1)	Reference	
Swickers Var	24 (25.8)	2.1 (0.7 - 6.3)	0.208
Swickers High	11 (20.0)	1.4 (0.4 - 5.1)	0.595

Table 10 (cont) Number and percentage of pigs with a positive result for each infectious agent by batch and Kingsley's pleurisy categories. Estimated univariable odds ratios for the association between pleurisy categories and the occurrence of a positive test result for each agent. Odds ratios were only estimated when there were at least five pigs testing positive. Bolded p values are overall likelihood ratio test p values. Non-bolded p values are individual Wald test p values

Infectious agent Variable/category	No (%) pigs positive	Odds ratio (95% CI)	p value
P. multocida L3			
Batch pleurisy score			**0.098**
≤ 10%	4 (5.3)	Reference	
>10%	20 (14.8)	4.0 (0.8 - 20.2)	0.098
Swickers pleurisy category			**0.137**
Swickers Low	2 (3.2)	Reference	
Swickers Var	15 (16.1)	7.4 (1.0 - 53.4)	0.047
Swickers High	7 (12.7)	5.3 (0.6 - 45.0)	0.128
P. multocida L6			
Batch pleurisy score			**0.618**
≤ 10%	6 (8.0)	Reference	
>10%	14 (10.4)	1.3 (0.4 - 4.4)	0.618
Swickers pleurisy category			**0.470**
Swickers Low	8 (12.9)	Reference	
Swickers Var	6 (6.5)	0.5 (0.1 - 1.7)	0.238
Swickers High	6 (10.9)	0.9 (0.2 - 3.2)	0.817
P. multocida NT			
Batch pleurisy score			
≤ 10%	2 (2.7)	-	
>10%	2 (1.5)	-	
Swickers pleurisy category			
Swickers Low	0 (0.0)	-	
Swickers Var	4 (4.3)	-	
Swickers High	0 (0.0)	-	
S. suis			
Batch pleurisy score			**0.342**
≤ 10%	38 (50.7)	Reference	
>10%	80 (59.3)	1.6 (0.6 - 4.4)	0.342
Swickers pleurisy category			**0.355**
Swickers Low	38 (61.3)	Reference	
Swickers Var	45 (48.4)	0.5 (0.2 - 1.7)	0.286
Swickers High	35 (63.6)	1.2 (0.3 - 4.3)	0.762
A. indolicus			
Batch pleurisy score			**0.150**
≤ 10%	1 (1.3)	Reference	
>10%	8 (5.9)	4.7 (0.6 - 38.0)	0.150
Swickers pleurisy category			**0.840**
Swickers Low	2 (3.2)	Reference	
Swickers Var	4 (4.3)	1.3 (0.2 - 7.6)	0.735
Swickers High	3 (5.5)	1.7 (0.3 - 10.8)	0.556

Table 10 (cont) Number and percentage of pigs with a positive result for each infectious agent by batch and Kingsley's pleurisy categories. Estimated univariable odds ratios for the association between pleurisy categories and the occurrence of a positive test result for each agent. Odds ratios were only estimated when there were at least five pigs testing positive. Bolded p values are overall likelihood ratio test p values. Non-bolded p values are individual Wald test p values

Infectious agent Variable/category	No (%) pigs positive	Odds ratio (95% CI)	p value
A. porcinus			
Batch pleurisy score			**0.419**
≤ 10%	5 (6.7)	Reference	
>10%	4 (3.0)	0.4 (0.0 - 3.5)	0.419
Swickers pleurisy category			**0.888**
Swickers Low	3 (4.8)	Reference	
Swickers Var	4 (4.3)	1.8 (0.0 – 33.9)	0.686
Swickers High	2 (3.6)	1.0 (0.0 – 25.9)	0.987
A. minor			
Batch pleurisy score			**0.873**
≤ 10%	4 (5.3)	Reference	
>10%	8 (5.9)	1.1 (0.3 - 4.3)	0.873
Swickers pleurisy category			**0.617**
Swickers Low	3 (4.8)	Reference	
Swickers Var	7 (7.5)	1.6 (0.4 - 7.0)	0.529
Swickers High	2 (3.6)	0.7 (0.1 - 5.0)	0.756
A. porcitonsilarum			
Batch pleurisy score			**0.430**
≤ 10%	3 (4.0)	Reference	
>10%	9 (6.7)	1.7 (0.4 – 6.5)	0.430
Swickers pleurisy category			**0.541**
Swickers Low	2 (3.2)	Reference	
Swickers Var	7 (7.5)	2.4 (0.5 - 12.2)	0.276
Swickers High	3 (5.5)	1.7 (0.3 – 10.8)	0.556
E. coli			
Batch pleurisy score			**0.714**
≤ 10%	12 (16.0)	Reference	
>10%	25 (18.5)	1.2 (0.4 - 3.8)	0.714
Swickers pleurisy category			**0.431**
Swickers Low	14 (22.6)	Reference	
Swickers Var	12 (12.9)	0.5 (0.1 - 1.6)	0.219
Swickers High	11 (20.0)	0.9 (0.2 - 3.3)	0.838
PCV2			
Batch pleurisy score			**0.717**
≤ 10%	28 (47.5)	Reference	
>10%	50 (41.7)	0.8 (0.2 - 3.0)	0.717
Swickers pleurisy category			**0.976**
Swickers Low	21 (42.0)	Reference	
Swickers Var	39 (43.8)	1.1 (0.3 - 5.0)	0.870
Swickers High	18 (45.0)	1.2 (0.2 - 7.4)	0.834

The point estimates of the odds ratios for the association between *A. pleuropneumoniae* and *P. multocida* L3 were also large, suggesting increased risk of positive test results for pigs from pigs with batch scores >10% and farms in the variable and high pleurisy categories. However, the estimates were imprecise, with 95% confidence intervals including one, so given the limited power of the study (due to small sample size and clustering of pathogens by farm) it is not possible to reach a definitive conclusion about this association. There was no evidence of a strong association between the identification of other bacterial species or PCV2 from pigs or the dichotomous plate count variables and the batch or farm pleurisy category (Table 10).

As it is likely that the presence of cranioventral pneumonia might confound the observed association between the identification of pathogens and the batch or farm pleurisy category, further analyses, adjusting for cranioventral lung score, were conducted where the outcomes were the identification of *M. hypneuomiae*, *A. pleuropneumoniae* and *P. multocida* L3. This was done using a variable that categorised the cranioventral lung score into three categories each containing approximately equal numbers of pigs. The point estimates were still large, but those for *M. hyopneumoniae* and *P. multocida* were smaller than the unadjusted estimates indicating that at least some of the observed crude association was due to confounding. The estimates were also less precise with confidence intervals including one in most cases so given the limited power of the study (due to small sample size and clustering of pathogens by farm) it is not possible to reach a definitive conclusion about these associations. (Table 11).

Table 11 Estimated odds ratios for the association between pleurisy categories and the occurrence of a positive test result for *M. hyopneumoniae*, *A. pleuropneumoniae* and *P. multocida* L3 after adjusting for CVP score (split into three categories). Bolded p values are likelihood ratio test p values for the variable. Non-bolded p values are individual Wald test p values

Infectious agent Variable/category	Odds ratio (95% CI)	p value
M. hyopneumoniae		
Batch pleurisy score		**0.066**
≤ 10%	Reference	
>10%	3.7 (0.9 - 16.0)	0.075
Swickers pleurisy category		**0.004**
Swickers Low	Reference	
Swickers Var	12.8 (2.4 - 68.8)	0.003
Swickers High	8.0 (1.2 - 51.5)	0.028
A. pleuropneumoniae		
Batch pleurisy score		**0.141**
≤ 10%	Reference	
>10%	5.3 (0.5 - 62.0)	0.184
Swickers pleurisy category		**0.089**
Swickers Low	Reference	
Swickers Var	1.3 (0.1 - 19.4)	0.852
Swickers High	8.2 (0.7 - 96.7)	0.096
P. multocida L3		
Batch pleurisy score		**0.209**
≤ 10%	Reference	
>10%	2.8 (0.5 - 15.7)	0.229
Swickers pleurisy category		**0.265**
Swickers Low	Reference	
Swickers Var	5.1 (0.6 - 42.6)	0.134
Swickers High	3.8 (0.4 - 38.1)	0.253

Looking at the resistance profile there also seems no association with the batch pleurisy score. The only noticeable resistance that occurs in the high pleurisy farms is the florfenicol resistance (Table 12).

Table 12. Antimicrobial sensitivity of *S. suis* isolates against five antimicrobials. SXT = Cotrimoxazole (trimethoprim/sulfamethoxacole)

Code for farm	Total pleurisy score (%) for batch sampled	Antibiotic senstivity of *Streptococcus suis* isolates				
		Ampicillin	Erythromycin	Florfenicol	Penicillin	SXT
0881	1.49	S	R	S	S	S
1012	1.75	S	S	S	S	S
0863	1.76	S	S	S	S	S
0884	1.79	S	R	S	R	S
0817	1.85	S	R	S	S	S
0882	1.99	S	R	S	S	S
0414	3.48	S	R	S	S	I
0862	3.8	S	R	S	S	R
0461	4.52	S	R	S	R	R
1055	5.88	S	R	S	S	R
1041	7.84	S	R	S	S	S
0822	7.94	S	R	S	S	S
0814	8.73	R	R	S	R	S
1021	9.3	S	R	S	S	S
0615	10.46	R	R	S	R	R
0462	11.93	S	R	S	S	S
1031	13.66	S	R	S	S	S
0443	14.52	S	R	S	S	S
0871	15.48	R	R	S	R	R
0611	16.46	S	R	S	S	I
0442	16.82	S	R	S	S	S
1011	17.89	S	R	S	S	S
891	19.58	S	R	S	S	S
1121	20.16	S	R	S	S	S
1053	23.31	S	R	S	S	R
0842	23.81	S	R	S	R	S
0448	23.94	S	R	S	S	S
1052	24.36	S	R	S	S	R
1054	25.45	S	R	S	S	I
0816	27.46	S	R	S	S	S
0447	30.26	S	R	R	S	R
0225	38.46	S	I	S	S	S
0211	40	S	S	S	S	S
1111	41.01	S	R	R	S	R
0111	45.81	S	R	S	R	S
0851	46.3	R	R	I	R	S
1051	46.73	S	R	S	S	S
0444	62.96	S	R	S	R	S

All isolates were sensitive to ceftiofur and all were resistant to tilmicosin and tulathromycin.

4. Application of Research

Pleurisy has a tremendous impact on the cost of the production and the cost of the slaughter process. Studies in England have shown that the herds with pleurisy prevalence running at >10% at slaughter experienced post-weaning mortalities rates around 3.3% higher than unaffected units. These studies have predicted that each 1% increase in pleurisy prevalence relates to a reduction of 1.55 – 2.5 g/day average lifetime weight gain at batch level or in other terms, each 1% increase in pleurisy prevalence at batch level resulted in 0.07 kg decrease in average slaughter dead weight per pig. In other words pleurisy is costing the producer a lot of money. To get a handle on the causes of pleurisy under Australian conditions, this study was undertaken. It is vital to understand the bacterial and viral species that are associated with chronic pleurisy.

For the farmer the knowledge gained on the bacterial species involved in pleurisy is useful and will ensure targeted investigations and the development of appropriate effective treatment and prevention programs.

The main points from this research are the importance of the two primary pathogen species in pigs with pleurisy, PCV2 and *M. hyopneumoniae*. As well, the importance of the secondary pathogens, which have so far been ignored in the fight against respiratory disease, was shown.

The outcomes achieved are the knowledge of the bacterial and viral species that are found in pigs with pleurisy at the abattoir and this knowledge will give farmers the opportunity to investigate if these species are controlled on their farm and implement control measures.

5. Conclusion

At slaughter, chronic pleuritis and cranio-ventral pulmonary consolidation are the most frequent observations in the lungs of pigs (Martinez et al., 2007). These consolidations and pleurisy are not associated with significant losses on the farms

due to growth retardation, pure feed efficiency and treatment cost, but chronic pleurisy also has a huge impact at the abattoir due to increased time for slaughter and compromised carcass weights and grades. Chronic respiratory disease represents a long-standing problem can lead to prolonged or permanent damage. Contrary to the acute outbreak, chronic disease is not normally caused by a single agent but by a variety of pathogens (Mark White 2012). While a number of recognised bacterial pathogens e.g. *S. suis*, *M. hypneumoniae*, *P. multocida* and *A. pleuropneumoniae*, as well as PCV2 were found in this study no single dominant infectious cause of pleurisy could be found.

The pathogens found in this study are similar to pathogens found in similar studies in France, Denmark, Norway, Canada and Brazil (Dutra et al 2013, Fablet et al 2012b, Høie et al 1991, O'Sullivan et al 2011, Tucker et al 2000). As in Canada, the most frequent pathogen found in the current study was *S. suis*. This was followed by *M. hyopneumoniae*, which was clustered by farm. Furthermore, after adjusting for cranioventral lung score, there was increased risk of identification of *M. hyopneumoniae* from pigs coming from farms in the variable and high farm pleurisy categories compared to farms in the low pleurisy category. A similar trend was seen in pigs coming from batches with a pleurisy score of >10% compared to those from batches where the pleurisy was ≤ 10%, but this estimate was less precise. *M. hyopneumoniae* has been found in the other countries as a major concern despite widespread vaccination (Opriessnig et al 2011) and it clearly remains a major concern in Australia as well, despite vaccination programs.

We found PCV2 was present in 44.1% of pigs in large numbers. It has been reported that PCV2 is underpinning pleurisy (Tucker et al 2000), which might be due to its potential to reduce acquired immunity to other pathogens (Oprieessnig et al 2006). Vaccination against PCV2 has been shown to decrease co-infection with secondary pathogens (Hansen et al 2010) and IgM antibodies against PCV are associated with reduced pleuritis or lung lesions at slaughter (Wellenberg et al 2010). It is well established that a combination of low pathogenic agents, such as PCV2 and *M. hyopneumoniae*, can result in severe respiratory disease (Opriessnig et al 2011).

There is a strong link between pleurisy and underlying pneumonia, a correlation which studies have relied on when sampling to identify the traditional bacterial contributors to respiratory disease, therefore, the presence of *P. multocida* in 21.4 % of the pigs in the current study is not surprising, as *P. multocida* has been found to be an important agent in the development of pneumonia (Dutra et al 2013). Experimental infections with *P. multocida* failed to cause disease, but, with the addition of *M. hypneumoniae,* severe coughing and extensive lesions could be observed (Opriessnig et al 2011). So the finding of many farms being co-infected with *M. hypneumoniae* and *P. multocida* might explain some of the high pleurisy cases. The same synergistic or additive effect has been found for *M. hyopneumoniae* and *A. pleuropneumoniae* (Opriessnig et al 2011). Overall synergistic effects of pathogens could explain the observation that most pigs had two pathogens. However, not all farms with high pleurisy had a combination of pathogens and one farm in particular stood out with a very high pleurisy percentage with only *S. suis* being retrieved from the lungs. The explanations might be other contributing factors such as parasites and non-infectious factors, such as the environment, management and pig factors. It has been found that parasitism can affect the ability to respond to respiratory disease and that it can also negatively impact *M. hyopneuomiae* vaccination (Opriessnig et al 2011). Factors such as air pollution due to ammonia and airborne particles can trigger inflammatory reactions and reduce the resistance to respiratory infections and have been attributed negatively with production efficiency (Banhazi 2013). Other risk factors for pleurisy have been identified such as no all-in-all out flow, rearing pigs in the same air space with an age difference of more than one month and repeated mixing of pigs (Jaeger et al, 2012)

A lot of bacterial species that are classed as species of minor relevance based on their ability to induce respiratory disease and lesions (secondary pathogens) (Opriessnig et al 2011), such as the *Actinobacillus* species, *M. hyorhinis*, *P. multocida* and *S. suis,* were found in this study. The finding of *S. suis* from 38 farms, with the organism being clustered by farm, and the observation that *S. suis* was present on plates with high plates score indicates that this pathogen was present in lungs in substantial numbers. The fact that it was on many farms with a high batch pleurisy score and that it was the only pathogen found in lungs from a batch with 38.5% pleurisy

suggest that it has a contribution to pleurisy. *S. suis* is widely regarded as a commensal in the upper respiratory tract and can be transmitted via vertical transmission, however the main route of transmission is the respiratory route (Amass 1996, Higgins & Gottschalk 2006). It is estimated that almost 100% of pig farms worldwide have carrier animals (Higgins & Gottschalk 2006). However, reports in the literature indicate that the gdh PCR can give false positives (Goyette-Desjardins et al 2014), which might have caused some over-estimation of the prevalence of this organism. In the current study an isolate was only identified as *S. suis* if two different PCR assays agreed, avoiding false positive diagnosis. Therefore, our finding of 83% of farms having pigs that have *S. suis* in lungs affected by pleurisy indicates that the statement of almost 100% of farms worldwide having carriers (Higgins & Gottschalk 2006) appears correct. *S. suis* is regarded as one of the most important pathogens in the porcine industry with many disease symptoms, but the most renown ones being septicaemia and meningitis (Goyette-Desjardins et al 2014).

Florfenicol resistance was only found in batches (four farms) with high lung score with the lungs also being positive for *M. hyopneumoniae*. These lungs also had either *P. multocida* (three farms) or *A. pleuropneumoniae* (one farm). One of the farms was associated with very high numbers of *P. multocida*. The *S. suis* resistance was used as an indicator for resistance levels of these farms. It is well known that resistance can be transmitted between bacterial species (da Costa et al 2013) and our assumption that resistance present in *S. suis* might reflect the resistance in other respiratory pathogens from this farms is a reasonable assumption. Resistance to florfenicol has been detected in Australian porcine *P. multocida* isolates at a level of 2%, while no resistance was found in *A. pleuropneumoniae* (Dayao et al 2014).

The *Actinobacillus* species identified by 16S rDNA sequencing, which were found on 28 out of 46 farms, are not believed to be associated with pathogenicity (Chiers et al 2001). However, as early as 2001 there was a hint that there are new species among the *Actinobacillus* species that might possibly be pathogenic (Kielstein et al 2001). In a recent a preliminary study performed in our laboratory, we have examined 36 field isolates of *Actinobacillus* species (many from the current pleurisy study) by *recN* sequencing and aligning the sequences to the reference strains for the

known *Actinobacillus* species. This preliminary study revealed that a total of 23 isolates could not be assigned to any of the *Actinobacillus* species. The phylogenetic tree of the *recN* sequences indicated the presence of at least one novel species. Given the uncertainity over identifaction and classification, it is not possible to be confident about the pathogenic potential of these isolates.
Many of the *P. multocida* isolates were recovered in larger numbers and might have been a main contributor to the observed pleurisy.

There were some plates that had a lot of bacteria on the plate but they were environmental contaminants among them *Streptococcus* species and some of unknown identity. Isolates that were Gram positive and had a colony appearance typical of environmental organisms were not further analysed. At the slaughter pigs were submerged in a scaling water tank and the possibility of contamination of the lungs with bacteria is a possibility. However, some of the pigs had no bacterial growth on their plates at all, despite the fact that the batches examined in this study never being the first ones to go through the tank. The fact that not all lungs from one batch had the same pathogens also speaks against contamination by the water. It is known that lung lesions normally prevent contaminations due to mucopurulent or catarrhal exudate in the airways of the affected pigs (Sorensen et al 2006). A study of specific pathogen free pigs without lesions in the lung produced negative PCR results for *M. hypneumoniae, A. pleuropneumoniae, P. multocida, H. parasuis* and *S. suis* when their lungs were examined after scalding (Marois et al 2008). Finally, any environmental contamination could have been due to the breakage of some tissue when removing adhered lungs.

M. hyopneuomia and *M. hyorhinis* are known pathogens in pigs, the pathogenicity of *M. flocculare* is still in question with the general belief being that it is not pathogenic (Kobisch and Friis 1996). However, a case study of the Faculty of Veterinary Medicine Timisoara in Romania found *M. flocculare* in lesions of enzootic pneumonia in younger pigs and therefore suggesting a pathogenic potential (Faur et al 2010).

As this study only looked at one batch per farm it has not included the seasonal variation. Seasonal variation of pleurisy have been observed in other studies (Eze et al 2015). It is known that *A. pleuropneumoniae* infections occur in spring and autumn and could affect the pathogens that might be involved in the respiratory disease of a particular batch and hence might not represent all the pathogens present on the farm.

Summary

Despite no single cause of pleurisy being identified in this study, the finding of 38 and 34 farms being positive for *S. suis* and *M. hyopneumoniae,* respectively, points out the importance of these bacterial species as has been noted in other countries (Opriessnig et al 201; Goyette-Desjardins et al 2014). There was an association between presence of *M. hyopneumoniae* and pigs with pleurisy coming from farms with high pleurisy classed farms compared to pigs with pleurisy coming from low pleurisy classed farms. However, given the design of the study it is not possible to infer that there is a causal relationship between *M. hyopneumoniae* and pleurisy. Also, as there were farms that were negative for *M. hyopneumoniae* but which had a high batch pleurisy score (three of 35 high batch pleurisy score farms) and also some farms that were positive for *M. hyopneumoniae* that had a low batch pleurisy score it is clear that *M. hyopneumoniae* is not specific to high pleurisy batches/farms.
The finding of a lot of pathogens regarded as secondary pathogens, some of which were in high numbers (*S. suis* and *P. multocida*) in some of the lungs, points to the importance of these pathogens in the porcine respiratory disease complex.
In light of synergistic interactions of bacteria/viral species, of which *M. hyopneumoniae* and PCV2 have been implicated as a common species, the finding of 74% of the farms with a positive result for *M. hyopneumoniae* and 63% of the farms with a high concentration of PCV2 is a concerning finding.

6. Limitations/Risks

This study was only done in Queensland and involved a limited number of farms. We believe that the results are most likely the same for other states in Australia, as Queensland farms are not markedly or obviously different to the rest of the

farms in Australia. As well, the pathogens seen in our reference diagnostic services submitted from Queensland farms are not different to the ones in the other states of Australia. However, it is important that the key limit of this study, the small sample size of farms, is recognized. This limitation was a reflection of the resources available to do the work.

7. Recommendations

The finding that *M. hyopneumoniae*, *P. multocida*, *S. suis* and PCV2 were most commonly identified and strongly clustered by farms is a major finding. *M. hyopneumoniae* and *S. suis* were present on 34 and 38 out of 46 farms, while *P. multocida* was present on 24 out of 46 and PCV2 on 29 farms of 39 farms tested. *Actinobacillus* species were also seen on 29 farms out of 46. This means that most of the bacterial species seen in pleurisy affected lungs were mainly those typically regarded as secondary pathogens.

In the light of synergistic interaction of *M. hyopneumoniae* and PCV2 with secondary pathogens, it seems vital that treatment and prevention/control options for *M. hyopneuomiae* and PCV2 are looked at again. The results of this study clearly show that farmers need to have a look at the status of these two pathogens on their farm and re-evaluate the treatment and prevention/control regimens currently in place.

It is well recorded in literature that *S. suis* is on most pig farms, but not much attention has been paid to this pathogen. The same can be said for *P. multocida* and *Actinobacillus* species. All are classed as secondary pathogens and therefore regarded as not very important. The results of this study suggest that it would be advisable to have a closer look at these "secondary" pathogens in terms of prevention and treatment options, be that early intervention or vaccination.

We are currently looking at defining the identification of the *Actinobacillus* species obtained in this study, as some of these have been associated with respiratory disease outbreaks and we have potentially new species among the isolates we currently identified as the "*A. minor*/*porcitonsillarum* complex". We

are planning to develop PCR based identification tools to help in understanding the role of these bacteria in respiratory disease and pleurisy.

So the main recommendations from this research are:

- The industry should consider how well PCV2 is controlled on pig farms.
- The industry should review *M. hyopneumoniae* vaccination control
- The industry should have a look at the level of *S. suis*, *P. multocida* and *Actinobacillus* species on farm level and at possible treatment/prevention to keep the level of these species down.

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