Australian Government
Department of Agriculture

AUSTRALIAN Pork™

Factors that impact on the expression of respiratory disease in pigs

Final Report
APL Project 2014/447

November 2015

NSW Department of Primary Industries
Dr Alison Collins
Elizabeth Macarthur Agricultural Institute
PMB 4008
Narellan NSW 2567

Acknowledgements

This project was supported by funding from Australian Pork Limited and the NSW Department of Primary Industries. Statistical support was provided by Ms Gemma Wyburn and Dr Damian Collins (NSW Department of Primary Industries). Technical support for the literature review was provided by Ms Gemma Wyburn and Ms Bethany Bowring (NSW Department of Primary Industries). Dr Graeme Eamens, formerly with NSW Department of Primary Industries generously provided all the raw data from APL project 1870 that was used to analyse the impact of multiple respiratory pathogens on variation in pig production.

Executive Summary

Both bacterial and viral pathogens cause respiratory disease in pigs, but the severity of respiratory disease also depends on environmental and host factors. The first part of this study focussed on the impact of potentiating or multiple pathogens, like *Mycoplasma hyopneumoniae*. The study highlighted the significant negative impact that multiple respiratory pathogens have on the expression of respiratory disease in growing pigs. Although infection with *M.hyopneumoniae* or *Pasteurella multocida* individually can affect production and carcase composition, pigs challenged with both of these pathogens were more severely affected with pneumonia (lung lesions and clinical signs), and also with reduced weights, ADG, feed intake and gain to feed ratios. Grower pigs infected with both pathogens suffered severe reductions in liveweight at 20 weeks of age (16kg lighter) relative to control pigs or pigs challenged with either pathogen alone (4-10kg lighter).

The present study also demonstrated that multiple pathogens significantly increased the variation in production measures within treatments, which affects profitability by increasing the number of 'tail-ender' pigs, disrupting all-in-all-out (AIAO) production flows and increasing days to slaughter. Multiple pathogens specifically increased variation in ADG, feed intake, feed efficiency and body composition (muscle and fat proportions), as well as the proportion of lung tissue affected with pneumonia. There was also a trend towards greater variation in slaughter weights in pigs infected with multiple pathogens.

Variation in market weight impacts on profitability by increasing housing costs per pig, due to delayed emptying of sheds for light weight pigs in AIAO production. To avoid variation in market weight, producers often sort pigs on weight before sale. However, frequent mixing of pigs can have significant impacts on both animal health and welfare. Producers can maintain AIAO production if they market different weight pigs to the most appropriate buyer. However, this is likely to cause additional sorting and transport costs.

The variation in feed intake, growth rates and weights caused by disease may be overcome with additional veterinary treatments or dietary formulations to help slow-growing pigs recover prior to sale. However, all of these strategies ultimately lead to increased production costs and reduced profitability, so cost-benefit analyses need to be performed.

Under commercial conditions, pigs may be constantly challenged by pathogens, and are unlikely to face a single respiratory infection at one time point, as occurred in this study. In addition, they may be exposed to poor environmental conditions and infections from enteric pathogens, all of which stimulate the pig's immune system and divert energy resources away from growth. It is therefore expected that pigs housed under commercial conditions, with multiple pathogen exposures and environmental stressors, would exceed the growth reductions seen in this trial. The AUSPIG modelling software could estimate the impact of increased variation under commercial conditions using the data from previous experimental challenge trials.

The second part of this study identified the key management practices associated with increased risk of disease outbreaks. The expression of respiratory disease is definitely exacerbated by multiple pathogens, but also by many other factors including high stocking density, poor air quality, poor ventilation, continuous production flows and frequent mixing of pigs, temperature fluctuations and the absence of cleaning and disinfection between batches. The literature quantifies the increased risk of respiratory disease associated with many of these management factors, with biosecurity and air quality

as two of the most important factors. Control or prevention of respiratory disease therefore relies on producers utilizing management tools to reduce these risks.

The efficacy of disease management tools need to be evaluated under commercial conditions so the most useful and timely health monitoring tools need to be identified for respiratory disease. Real-time monitoring of disease is especially important to provide producers with the timely advice to prevent disease. Coughing is a useful clinical sign of a range of respiratory diseases, but requires pathogen-specific detection to indicate the cause of current disease. Although measuring lung lesions at slaughter is a common tool used to quantify the impact of respiratory disease, the information is not provided in real-time, it is not pathognomonic for specific diseases, and under-estimates the extent of damage because lesions resolve before slaughter. Serum IgG antibodies are a useful indicator of pathogen-specific infections at the herd level; however, serum IgG indicates past, not current infection. The detection of pathogen-specific IgA in oral fluids provides an earlier indicator of infection than serum IgG, and oral fluid samples are a cost-effective way to determine the timing of infection in pens of pigs. Oral fluid testing for either antibodies or pathogens has the potential to facilitate surveillance and detection of disease in a population.

Quantifying the numbers or 'load' of pathogens in known volumes of air may be a useful indicator of disease expression at the herd level, and can identify the presence of more than one pathogen, but field studies are still needed to correlate pathogen loads with other disease scores (coughing, serology or subsequent lung lesions at slaughter) and production. Future research may identify threshold levels for different pathogens that correlate with increased severity of respiratory disease. Focussing on quantifying potentiating pathogens, such as *M.hyopneumoniae*, influenza A virus and PCV2, may also be important for predicting potential respiratory disease outbreaks. However, it is likely that more than one monitoring tool is needed to quantify the impact of respiratory disease on farm.

Our research outcomes are already in use by a vaccine company working with commercial herds to control coughing. We plan to collaborate with the University of Melbourne to identify the most quantitative and cost effective diagnostics for measuring the impact of respiratory disease.

Table of Contents

List of Tables

1. Background to Research

Problems and needs arising from the research:

The third objective of this project was to use the CT scanner to measure the composition of primal cuts and investigate the impact of single or multiple respiratory pathogens on variation in composition and size of primal cuts. As previously notified, it was not possible to transfer the data from the obsolete magnetic tapes to the new software to allow us to quantify the impact of multiple pathogens on variation in the composition of primal meat cuts. However, we did discuss the option of collaborating with John Black to incorporate the variation data collected from this trial to estimate the cost of multiple respiratory pathogens in grower/finisher pigs using AUSPIG.

Opportunities from the research:

Both pig producers and veterinarians are often reluctant to replace antibiotics with vaccines for disease control, as it's often difficult to demonstrate the efficacy of vaccines to improve productivity and control of disease, especially with multifactorial disease complexes like respiratory disease. The outcomes of this project have helped us identify the optimal samples and assays to measure the impact of respiratory diseases on pig health and production. The project has also identified the relative risk of respiratory diseases under different management practices. This knowledge has recently proved useful for Boehringer Ingelheim, who are implementing a swine cough control program in combination with the release of a new *M.hyopneumoniae* vaccine in Australia. However, the knowledge gained from this APL funded project could apply equally to other vaccine companies and against other causes of respiratory disease.

We have used the knowledge gained from the literature review to expand the swine cough control program developed by Boehringer Ingelheim to record additional data from farms on management factors that may impact on the prevalence or severity of clinical disease and production losses in affected herds. More specifically this includes recording variation in P2 backfat measures at slaughter, the use of aerosol disinfection within production batches, measuring air quality in sheds and the proportion of 'tail-ender' pigs before and after vaccination. NSW DPI will be providing support for this disease management program by testing sera for IgG to *M.hyopneumoniae*, by quantifying numbers of *M.hyopneumoniae* and other respiratory pathogens in affected lung tissue and by providing histopathology support for the differential diagnosis of *M.hyopneumoniae*-induced pneumonia.

The review of diagnostics and clinical signs to measure the severity of respiratory disease in this project has led to collaborative research discussions with Boehringer Ingelheim on ways to measure the efficacy of their *M.hyopneumoniae* vaccine to control coughing in commercial herds. Boehringer Ingelheim have agreed to collect oral fluid and air samples from herds with clinical evidence of pneumonia, especially coughing. We have already investigated the detection of *M.hyopneumoniae* DNA in oral fluid samples and found the sensitivity of the test to be very low. We intend to apply for a collaborative project between NSW DPI, the Pork CRC and Boehringer Ingelheim to develop an ELISA for the detection of *M.hyopneumoniae* specific IgA in oral fluids, and to correlate IgA levels with other measures of respiratory disease including serum IgG titres to *M.hyopneumoniae*, pig growth, coughing scores and slaughter lesions. Preliminary research suggests that pigs infected with *M.hyopneumoniae* develop IgA antibodies in oral fluids close to the time of clinical disease and production losses and 4 weeks earlier than serum IgG responses. The objective of this research is to find a herd based and inexpensive assay for the earlier diagnosis of pneumonia in commercial herds, to ultimately reduce production losses.

We are also investigating a collaborative project between the University of Melbourne, the Pork CRC, NSW DPI and Boehringer Ingelheim to correlate pathogen loads in air samples collected from commercial piggeries with other measures of respiratory disease expression including clinical disease, IgG and IgA antibodies to *M.hyopneumoniae* and production losses.

Quantify potential benefits for the industry and possible implications of the research:
Improved control of respiratory diseases is likely to have significant economic benefits in increasing pig growth, improving feed efficiency and reducing the costs of treating sick pigs. Left untreated, respiratory disease can cost A$2.80 per pig, assuming 40% of lungs are affected with pneumonia at slaughter and ADG losses range from 12.7 to 15.9% in grower pigs and feed efficiency losses are about 13.8% (Pointon et al., 1985).

The staggering 16kg reduction observed in the mean weight of pigs challenged with both *M. hyopneumoniae* and *P. multocida* relative to 20 week old control pigs in this trial illustrates that there are significant economic costs associated with respiratory disease. These costs include the need for extra feed and time to allow these pigs to 'catch up' and reach market slaughter weights. Assuming ADG remains at 0.8kg/day, pigs challenged with multiple pathogens would require an additional 20 days to recover the lost 16kg, reducing profits by $12.60 per pig (assuming feed costs $300/tonne and pigs continue to eat 2.1 kg feed per day). Alternately, if the producer chose to sell pigs below market weight to maintain production flows, a 16kg weight reduction in hot carcass weight would lead to reduced profits of $56 per pig (350c/kg x 16 kg). Over a batch of pigs this would equate to large losses, even if the pigs challenged with multiple pathogens had recovered completely after 17 weeks of age. While pigs challenged with either *M. hyopneumoniae* or *P. multocida* alone also suffered reduced market weights (4 or 10kg respectively), the added feed costs to get these pigs to market weight would be substantially less ($3.15 to $7.88 per pig respectively).

This is the first study to account for the additional costs associated with increased variation in production caused by respiratory diseases. Variation in ADG and feed intake lead to an increased proportion of 'tail-ender' pigs, whereas variation in carcass size, shape and composition lead to increased processing costs. Variation in market weight leads to decreased profitability by increasing housing costs per pig because of delayed emptying of sheds. These additional effects of respiratory disease were not quantified in this study, but could be if the data was included in AUSPIG. The economic benefits of improved respiratory disease control by vaccination, medication or eradication (Swiss depopulation) could also be estimated in AUSPIG.

Reducing losses associated with respiratory diseases relies on implementing disease prevention strategies and also effective disease monitoring tools. The project's literature review identified biosecurity and hygiene as critical factors in reducing the incidence and severity of respiratory diseases. Management practices important in reducing respiratory disease included all-in-all-out production flows, minimising mixing of pigs, improving air quality, reducing stocking density, improving temperature control, cleaning and disinfection between batches of pigs and utilising prevention strategies like vaccination and disease eradication (Table 1).

Table 1: Summary of management factors that increase the risk and/or severity of respiratory disease.

Management factor	Consequence
Introducing small number of new stock from herds with lower health status	2.38 times increased risk of disease
Introducing 10-100 new animals	4.10 times increased risk of disease
Introducing more than 100 animals	6.89 times increased risk of disease
Ammonia @ 50 ppm	12% reduction in growth
Ammonia @ 100 ppm	30% reduction in growth
No ventilation	1.55 times increased risk of disease
Continuous flow production	Increased risk and severity of disease
Stocking density for finishers < $0.7m^2$/pig	Increased risk and severity of disease
Air space < $3m^3$/pig	Increased risk and severity of disease
Draughts in pens and temperature fluctuations	Increased severity of clinical signs and reduced ADG
Presence of multiple pathogen	Increased severity of disease

Pathogen load, pathogen virulence the host's immune response and the presence of potentiating or multiple pathogens can all be impacted by these management factors. However, the host's genetics also play a significant role in the immune response to respiratory pathogens. Respiratory disease expression can be measured indirectly by monitoring weight gain and feed intake in the grower/finisher period, variation in final weights and carcase composition, or more directly by measuring pathogen load, pro-inflammatory cytokines, specific antibodies and gross or histopathology lesions at slaughter. However, the most promising disease monitoring tools for the future include quantification of pathogen loads in air in real time, IgA detection in oral fluids and coughing scores.

2. Objectives of the Research Project

1. Complete literature review outlining factors that impact on respiratory disease expression, specifically researching the effect of multiple pathogens, pathogen load, air quality, the pro-inflammatory response and vaccination strategies to reduce the impact of pneumonia on pig growth, carcass yield and variation in weights and carcass composition.

2. Re-analyse the CT data from APL project #1870 to investigate the impact of single or multiple respiratory pathogens on variation in slaughter weight, feed intake and variation in whole carcass composition.

3. Use the CT scanner to measure the composition of primal cuts and investigate the impact of single or multiple respiratory pathogens on variation in composition and size of primal cuts.

3. Introductory Technical Information

In APL project 1870, Dr Graeme Eamens demonstrated that concurrent respiratory infections in pigs can increase the severity of pneumonia and cause complications which delay recovery from infection. Pigs infected with *Mycoplasma hyopneumoniae* prior to a *Pasteurella multocida* challenge suffered significantly more severe reductions in weight gain, feed intake and liveweight at 20 weeks of age (16kg lighter) relative to control pigs or pigs challenged with either pathogen alone (4-10kg lighter). This trial also demonstrated that the combined infection had significantly greater impacts on pneumonia severity (lung lesion and clinical scores), lethargy, and body composition in the grower and finisher phases. Pigs affected with pneumonia had reduced P2 fat, P2 muscle and % body fat (by weight), but increased % body muscle (by weight) over the whole carcase. The differences in body composition in specific primal cuts were not quantified in this trial. While significant variation in disease parameters was shown within treatments (uninfected, single infection or combined infections), the study didn't investigate whether the coefficient of variation was significantly different between treatments. Additional analysis was needed to determine whether concurrent respiratory infections increased variation in production measures, carcase traits and slaughter weights.

The most common respiratory diseases in Australian pig herds are enzootic pneumonia and pleuropneumonia, caused by *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae.* Although secondary or opportunistic pathogens such as *Pasteurella multocida, Mycoplasma hyorhinis, Haemophilus parasuis, Streptococcus suis, Actinobacillus suis, Arcanobacterium pyogenes* and *Escherichia coli* can also cause problems. A significant amount of research has been published on multiple infections associated with respiratory disease that suggests primary pathogens are generally viruses or mycoplasmas, with bacteria commonly infecting as secondary pathogens. Primary pathogens can predispose the pig to secondary pathogens by damaging the mucociliary apparatus or by suppressing the local or systemic immune response. Prior infection with *M.hyopneumoniae* is believed to increase the host's susceptibility to colonisation by *Pasteurella multocida* (Amass et al., 1994; Ciprian et al., 1988; Eamens et al., 2007) and *A.pleuropneumoniae* (Ciprian et al., 1994; Marois et al., 2009). *M.hyopneumoniae* infection also potentiates infection by porcine circovirus type 2 (PCV2), PRRS and swine influenza virus (Deblanc et al., 2013; Opriessnig et al., 2004; Shibata et al., 1998; Thacker et al., 1999; Thacker et al., 2001; Thanawongnuwech et al., 2004; Zhang et al., 2011). Many of these studies have highlighted the increased severity of clinical disease and innate immune responses associated with multiple pathogens. However, few have focussed on measuring the impact of multiple pathogens on variation in production and carcass measures.

The presence of multiple pathogens, pathogen load and virulence, as well as environmental factors (air quality, temperature, and season) all impact on respiratory disease expression. However host factors also play a significant role. The pig's genetics and immune response to pathogen colonisation can alter susceptibility to pathogens, but can also influence the host's ability to recover from disease. Relatively high heritability's have been published for disease resistance to enzootic pneumonia, pleurisy, Glässers disease and atrophic rhinitis. In addition, a number of innate and humoral immunity traits that are important in tolerating and recovering from infection are heritable. These will be studied in more detail in the literature review on factors that impact on the expression of respiratory disease. We already know that stimulation of the immune system impacts on the pig's metabolism, as nutrients and energy are sequestered away from muscle deposition for the production of acute phase proteins, antibodies and cytotoxic and regulatory T cells to help fight infection (Black and Pluske, 2011). In particular it is the release of pro-inflammatory cytokines, causing fever, anorexia and lethargy, which impact on the pig's metabolism.

Monitoring the impact of respiratory diseases is confounded by the multifactorial nature of disease and the difficulty in selecting a real time measure of pathogen load, immune response and disease severity that could alert producers to potential production losses before they occur. Many studies have measured pathogen loads, innate and humoral immune responses, real time lung lesion scores and clinical signs in pigs experimentally challenged with single or dual pathogens, in clean environmental conditions, far removed from commercial production. Measuring respiratory disease expression in commercial herds is complicated by non-specific clinical signs, genetic variation in respiratory pathogens reducing the specificity and sensitivity of serological and DNA detection assays (polymerase chain reaction), and the poor correlation between gross lung lesions at slaughter and the lifetime impact of pneumonia on the pig's production. Collection of broncho-alveolar lavage (BAL) fluids for innate and humoral antibody responses and quantification of pathogen loads are not feasible at the herd level, so substitutes need to be found. Real time monitoring of lung lesions with radiography, ultrasound or computer tomography is also unrealistic in commercial herds. There is minimal literature that investigates respiratory disease expression at the herd level, so this project will review the published studies into components of disease expression could help guide future research priorities into optimal samples and diagnostic assays for measuring respiratory disease expression in real time at the herd level. The ultimate aim of this work is to search the literature for immune markers or pathogen loads that correlate with pneumonia and the associated production losses to provide real time monitoring of respiratory health or disease at the herd level.

4. Research Methodology

4.1 Investigate the impact of single or multiple respiratory pathogens on variation in slaughter weight, feed intake and variation in whole carcass composition.

4.1.1 Experimental Design

Production and disease data were compiled from the previous trial (APL project #1870) consisting of pig weights, feed intakes, % lungs affected with pneumonia, % fat and muscle weight as a percentage of total body weight and P2 fat and muscle depths. This data was re-analysed to test the hypothesis that multiple respiratory infections cause greater variation in production and carcass traits than a single infection with either pathogen or no pathogen.

APL study #1870 was based on four treatment groups of approximately 16 grower pigs (10 weeks age) per treatment, maintained in individual pens within a controlled environment facility at EMAI. Pigs in groups 3 and 4 were challenged intratracheally with a culture of *M. hyopneumoniae* (Mhp) (field strain) at 11 weeks of age. Three weeks later, pigs in groups 2 and 4 were challenged intratracheally with *P. multocida* (Pm). Group 1 pigs remained as unchallenged controls (Table 2). Weights and feed intake were recorded weekly for individual animals. All pigs were scanned by computed tomography (CT) at three time points: initially prior to *P. multocida* challenge and then 2.5 to 3 weeks after challenge (16-17 weeks age) and 5 to 6 weeks after challenge (19 to 20 weeks age).

Table 2. Summary of experimental design

Group #	# pigs	+ Mhp @ 11 wks	+ Pm @ 14 wks
1	16	No	No
2	16	No	Yes
3	15	Yes	No
4	17	Yes	Yes

4.1.2 Production data

Using the original weight and feed intake data, average daily gain (ADG) and average daily feed intake (ADFI) were calculated for the 3 weeks post Mycoplasma challenge and the 4 weeks post Pasteurella challenge, as well as total weight gain to slaughter (10 to 24 weeks of age). Feed efficiency was calculated as gain to feed (rather than feed to gain) to allow the comparison of results on a unidirectional scale, as pigs suffered significant weight losses during the trial. Profitability will increase as gain to feed increases i.e. higher gain to feed occurs when less feed is needed for the same weight gain.

4.1.3 Carcass composition

The CT scans of all pigs were processed to reconstruct 3D measurements of total lung volume, pneumonia affected lung volume, and the volume of carcass muscle, fat and bone. Known tissue densities were used to convert tissue volumes to tissue weights. The accuracy of the calculated CT tissue weights were confirmed by calculating the total combined CT tissue weights for each pig and comparing this to the actual weight of the pig on the day of scanning. The percentage of each tissue type was then determined by dividing the specific tissue weight by the total pig weight. Pneumonia affected lungs had a different density than healthy lungs as measured by the CT. The percentage of

pneumonia lesions was calculated by dividing the volume of affected lung by the total volume of lung tissue.

4.1.4 Analysis of the data

During the Mycoplasma infection period, pigs were effectively in two treatment groups with two replicates of each treatment. Group 3 and 4 pigs were challenged with Mhp and pigs in groups 1 and 2 were uninfected controls. The effect of multiple pathogens was only analysed from 14 to 20 weeks of age.

Mean differences between treatments, as well as variation within and between treatments are presented in the results, although mean treatment differences were published previously (Eamens et al., 2007). Unbalanced analysis of variance (ANOVA) was preformed to determine if treatment affected the mean production and carcass traits in both periods (GenStat 17th Edn). Bartlett's test was used to determine if significant variation between treatments was present (GenStat 17th Edn). Significant treatment differences were identified through least mean difference analysis (means) or f-tests (variance) (Excel). Dot histograms of each production and carcass measure were created to visualise differences in variation and to ascertain whether data was normally distributed, or required transformation.

Lung lesion data was transformed ($\log_{10} + 0.1$) to stabilise the variance as in the original analysis. We used the log transformed lung lesion data for the Bartlett's test as this test is sensitive to departures from normality. If the data is from a non-normal distribution, Bartlett's test may be testing for non-normality rather than significant differences in variation.

One pig was removed from the trial data as it showed relatively poor weight gain throughout the whole trial (start weight 46kg, finish weight 64kg). Clinical records showed this pig to show some evidence of depression and mild anorexia during the second week of the Pasteurella infection period (15 weeks of age) but minimal evidence of lung damage was demonstrated with the CT scanner. This pig was obviously ill, but as there was no evidence that this was due to respiratory disease, so it was removed from the trial.

4.2 Literature Review: Factors that impact on the expression of respiratory disease and how to monitor them

The published literature was reviewed to identify risk factors associated with respiratory diseases, along with host responses to single or multiple pathogens and environmental factors that exacerbate disease. From the literature, the most important parameters for respiratory disease expression were identified. Physiological responses to infection, such as pathogen load, pathogen virulence, the humoral and innate immune response to infection and the presence of multiple pathogens were examined to identify their impact on variation in disease expression. Management practices that impacted on respiratory diseases were also investigated including production flows, cleaning and disinfection, ventilation, air quality (dust and ammonia), stocking rates, mixing of pigs, introducing new pigs, fluctuating temperatures and season were also studied with respect to respiratory disease expression. Although genetics almost certainly impacts on disease expression, its impact was more difficult to quantify. The multifactorial nature of respiratory disease means that a combination of pathogen load,

clinical disease, immune response and air quality may need to be measured to estimate the impact of respiratory diseases on farm.

This literature review focussed on the bacterial pathogens and diseases of major importance to Australia including the causes of enzootic pneumonia (*M.hyopneumoniae*) and pleuropneumoniae (*A.pleuropneumoniae*), with less emphasis on the causes of pneumonic pasteurellosis (*P.multocida*), and Glässers disease (*H.parasuis*).

This project also investigated how diagnostic assays developed to monitor respiratory disease expression in experimental challenge trials could be modified to monitor disease at the herd level using samples and techniques that are cost-effective and realistic in commercial pig herds. Measuring respiratory disease expression in commercial herds is complicated by non-specific clinical signs, serological and DNA detection assays (polymerase chain reaction) with poor sensitivity and specificity, and the underestimation of lung damage at slaughter due to lung tissue recovery in the intervening weeks. Antibody responses and pathogen loads need to be measured in clinical material other than broncho-alveolar lavage (BAL) fluid, due to the difficulty in collecting it. The literature review investigated air samples and oral fluids as alternative samples for antibody detection and quantification of pathogen loads. There is minimal literature that investigates respiratory disease expression at the herd level, so this project will review the published studies into components of disease expression that could help guide future research priorities into optimal samples and diagnostic assays for measuring respiratory disease expression in real time at the herd level. The ultimate aim of this work is to search the literature for immune markers or pathogen loads that correlate with pneumonia and the associated production losses to provide real time monitoring of respiratory health or disease at the herd level.

5. Results

5.1 Investigate the impact of single or multiple respiratory pathogens on variation in slaughter weight, feed intake and variation in whole carcass composition.

5.1.1 Weekly weights, average daily gain and slaughter weights

Pigs challenged with Mhp did not have significantly different mean weights or variation in weights in the 3 weeks post Mhp challenge (data not shown for 11 to 14 weeks). Subsequently, pigs challenged with both Pm and Mhp were significantly lighter than all other treatments at 16 and 18 weeks of age (Table 3). At 18 weeks of age (week 4 post Pm challenge), Mhp+Pm pigs were 12kg lighter than control pigs (Table 2). This did not translate into significant variation in pig weights between treatments, although there was a trend for greater variation in the Pm group at 17 weeks (P=0.143, not shown).

The dramatic impact of multiple pathogens on pig weight was reported previously (APL report # 1870), but was even larger at 20 weeks of age, when pigs challenged with both Pm and Mhp were 16kg lighter than controls. *P.multocida* challenged pigs were 10kg lighter and Mhp challenged pigs were 4kg lighter than controls.

Table 3: Mean pig weights (kg) post Pasteurella challenge for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	14 weeks	15 weeks	16 weeks	17 weeks	18 weeks
1 – Control	46.06[a]	52.2[a]	59.34[a]	65.35[a]	72.68[a]
2 – Pm	46.36[a]	45.95[b]	53.24[b]	59.27[a, b]	67.7[a]
3 – Mhp	47[a]	52.2[a]	57.92[a, b]	61.8[a, b]	70.77[a]
4 – Mhp+Pm	44.79[a]	42.58[b]	47.02[c]	55.14[b]	60.53[b]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

The most significant impact of Pasteurella infection on ADG occurred in the first week post Pm challenge (Table 4, week 14). The mean ADG for multiple pathogens was significantly lower than all other treatments at this time (P<0.001). The impact of the multiple infections continued for another week, relative to the control and Pm only treatments, but surprisingly the Mhp+Pm treatment showed a significantly greater mean ADG than all other treatments in week 3 post Pm challenge (Table 4).

Variation in ADG was also significantly greater in the first week post Pm challenge, with both groups of Pasteurella challenged pigs having greater variation than control or Mhp only pigs (Table 5). All infected pigs showed more variation in ADG than the control pigs at week 3 post challenge, but by week 4 there was no significant difference in ADG variation between treatments. There was no difference in variation between a single Pm infection and a combined infection (Mhp+Pm) in any week.

M.hyopneumoniae challenge did not significantly reduce mean ADG, but there was a trend towards reduced ADG at 2 weeks post challenge (P = 0.132). Mhp challenge did not increase variation in ADG either, but increased variation was observed in control pigs at 13 weeks of age (not shown).

Table 4: Average daily gain (kg) post Pasteurella challenge for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	14 weeks	15 weeks	16 weeks	17 weeks
1 – Control	0.887[a]	1.001[a, b]	0.859[a]	1.047[a]
2 – Pm	-0.058[b]	1.042[b]	0.862[a]	0.875[a, c]
3 – Mhp	0.743[a]	0.814[a, c]	0.557[b]	1.281[b]
4 – Mhp+Pm	-0.316[c]	0.635[c]	1.161[c]	0.77[c]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

Table 5: Bartlett's test for significant variation in pig average daily gain between treatments

Treatment	14 weeks	15 weeks	16 weeks	17 weeks
Control	0.031[a]	0.054[a]	0.022[a]	0.047[a]
Pm	0.21[b]	0.113[a]	0.108[b]	0.043[a]
Mhp	0.034[a]	0.014[b]	0.077[b]	0.104[a]
Mhp+Pm	0.177[b]	0.123[a]	0.112[b]	0.064[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

At 17 weeks of age, pigs challenged with multiple pathogens were on average gaining 277g per day less than control pigs. Pigs challenged only with Pm gained on average 172g less than control pigs. Interestingly, the Mhp pigs made a strong recovery in ADG at 17 weeks and were gaining 234g per day more on average than the control pigs (Table 4).

Somewhat surprisingly, pigs challenged with Mhp alone had significantly higher total weight gain and average daily gain over the whole trial period, compared with control pigs or pigs challenged with both pathogens (Table 6a). The unexpected transmission of *P.multocida* to control pigs in the last weeks prior to slaughter may help explain why Mhp pigs had higher growth rates than the control pigs over the whole trial period.

Table 6a: Mean slaughter weights and weight gains for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	# of pigs	Slaughter weight (24 weeks)	Gain Mhp period (10-13 weeks)	Gain Pm period (14-24 weeks)	Total Gain	Gain per day (whole trial)
1 – Control	14-16	100.9[a]	22.14[a]	53.55[a]	76.17[a]	0.794[a]
2 – Pm	15-16	103.9[a]	22.08[a]	56.49[a]	79.13[a, b]	0.824[a, b]
3 – Mhp	15	110.7[a]	23.06[a]	63.7[b]	86.76[b]	0.904[b]
4 – Mhp+Pm	16-17	100.7[a]	20.9[a]	55.33[a]	76.48[a]	0.797[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

There was no significant difference in variation of slaughter weights or total weight gains between treatments, however pigs infected only with Mhp showed a trend for decreased variation in their

slaughter weights (P=0.136). There was also a trend for Mhp infected pigs to have less variation in weight gain at 10 to 13 weeks (P=0.181) as well as over the whole trial (P=0.131). Any differences in slaughter weights between treatments may have been masked due to the unexpected transmission of *P.multocida* to the control pigs in the weeks prior to slaughter, which resulted in the death of two pigs in this group.

5.1.2 Average daily feed intake and gain to feed

M.hyopneumoniae challenge did not significantly reduce mean ADFI, however there was a trend towards reduced ADFI in Mhp challenged pigs at 12 weeks of age (P=0078, data not shown). However, significant variation in ADFI between treatments was observed at 12 and 13 weeks of age (Table 6b). Variation in ADFI increased significantly in the control pigs at 12 weeks and persisted to 13 weeks. Surprisingly, significantly reduced variation in feed intake was observed in Mhp challenged pigs at 13 weeks, but as there were significant differences between the two Mhp replicates (Mhp and Mhp+Pm), this variation is more likely to be due to environmental causes rather than the effect of the disease.

Table 6b: Bartlett's test for significant variation in average daily feed intake between treatments

Treatment	11 weeks	12 weeks	13 weeks
Control	122,229[a]	250,762[a]	221,464[a, c]
Pm	199,543[a]	89,287[b]	124,603[a]
Mhp	97,166[a]	64,130[b]	45,220[b]
Mhp+Pm	117,689[a]	96,708[b]	149,690[c]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

The most significant impact of Pasteurella infection on ADFI occurred in the first week post Pm challenge. The mean ADFI for Pm infected groups were significantly lower than control and Mhp only treatments at this time (P<0.001). Pigs challenged with multiple infections showed significantly reduced mean ADFI compared to all other treatments at 15 weeks and continued to be significantly lower than the control pigs (Table 7).

Table 7: Mean average daily feed intake (g/day) post Pasteurella challenge for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	14 weeks	15 weeks	16 weeks	17 weeks
1 – Control	1,925[a]	2,214[a]	2,280[a]	2,583[a]
2 – Pm	1,059[b]	1,916[a]	2,024[a, b]	2,390[a, b]
3 – Mhp	1,808[a]	2,054[a]	1,954[b]	2,252[b]
4 – Mhp+Pm	870[b]	1,227[b]	1,803[b]	2,144[b]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

Variation in ADFI was significantly greater in the second and third week post Pm challenge, with both groups of Pasteurella challenged pigs having greater variation than control or Mhp only pigs (Table 8). However, by 17 weeks of age, there was no difference in variation between treatments. Variation in

ADFI was not significantly different between pigs challenged with Pm alone or challenged with both pathogens at any week.

Table 8: Bartlett's test for significant variation in pig average daily feed intake between treatments

Treatment	14 weeks	15 weeks	16 weeks	17 weeks
Control	148,904[a]	90,380[a]	37,247[a]	131,973[a]
Pm	278,094[a]	317,252[b]	301,363[b]	156,466[a]
Mhp	58,163[b]	47,491[a]	37,471[a]	91,947[a]
Mhp+Pm	141,333[a, b]	360,984[b]	151,443[b]	202,094[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

After Pm challenge, Mhp+Pm pigs ate 745g (46%) less than they had the week before. Pigs challenged with Pm only also ate 799g (43%) less than they had the previous week before infection, while both control and Mhp only pigs increased their feed intakes in the same time (Table 9). By 17 weeks of age pigs infected with multiple pathogens were still eating less (439g/day) feed than the control pigs. Mhp only pigs were eating 331g less and Pm only pigs were eating 193g less on average per day than the controls (Table 7). Vets trying to medicate sick pigs in feed need to take into account this significant reduction in feed intake following Pasteurella infection.

Table 9: Change in average feed intake after Pasteurella challenge

Treatment	Average feed intake 13 weeks	Average feed intake 14 weeks (after Pm challenge)	Percentage change
Control	1598	1925	+20.5%
Pm	1858	1059	-43%
Mhp	1665	1808	+8.6%
Mhp+Pm	1615	870	-46.1%

In the period following Mycoplasma challenge, there were significant differences between gain to feed means at 11 and 12 weeks of age, but as these were between replicates, it is likely that environment played a role in these differences. Variation in gain to feed measures during Mhp infection could not be attributed to the Mhp challenge for the same reason.

The most significant impact of Pasteurella infection on gain to feed occurred in the first week post Pm challenge (14 weeks). The mean gain:feed for Pm infected groups were negative (ie. the pigs lost weight) and were significantly lower than control and Mhp only treatments at this time (P<0.001). However, *Pasteurella* challenged pigs appeared to increase gain to feed ratios quickly, with no significant difference between treatments at 15 weeks (Table 10). Surprisingly, Mhp+Pm pigs showed significantly higher gain to feed relative to all other treatments at 16 weeks, showing a rapid recovery from their weight loss at 14 weeks. Both groups of pigs challenged with Pasteurella showed reduced gain to feed over the 4 week post infection period (Table 10).

Table 10: Mean gain to feed post Pasteurella challenge for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	14 weeks	15 weeks	16 weeks	17 weeks	Total period
1 – Control	0.471[a]	0.456[a]	0.381[a]	0.411[a]	0.426[a]
2 – Pm	-0.591[b]	0.544[a]	0.348[a]	0.37[a]	0.38[b]
3 – Mhp	0.414[a]	0.399[a]	0.285[a]	0.582[b]	0.425[a]
4 – Mhp+Pm	-0.672[b]	0.476[a]	0.645[b]	0.368[a]	0.364[b]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

Variation in gain to feed was also significantly greater in both groups of Pasteurella challenged pigs in the first week post infection, which persisted throughout the trial for multiply challenged pigs, but only to 16 weeks for Pm only pigs (Table 11). Over the four week period, multiple infections lead to greater variation in gain to feed than control and Pm only pigs.

Table 11: Bartlett's test for significant variation in pig gain to feed between treatments

Treatment	14 weeks	15 weeks	16 weeks	17 weeks	Total period
Control	0.011[a]	0.009[a, b]	0.007[a]	0.003[a]	0.0014[a]
Pm	1.971[b]	0.013[a]	0.166[b]	0.005[a]	0.002[a]
Mhp	0.01[a]	0.004[b]	0.018[c]	0.022[b]	0.0022[a, b]
Mhp+Pm	0.842[b]	0.053[c]	0.011[a, c]	0.013[b]	0.0054[b]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

5.1.3 Carcass composition – Lung lesions

Pigs challenged with Mhp (groups 3 and 4) had significantly pneumonic lesions than uninfected pigs (groups 1 and 2) at the first scan (3 weeks post Mhp challenge), with only five control pigs showing lung lesions (less than 2% affected) (Table 12, Scan 1). There was also significantly more variation in the percentage of pneumonia affected lung in the Mhp challenged groups, with significant differences in variation seen between the two Mhp replicates (groups 3 and 4) at scan 1 (Table 13, Scan 1).

Table 12: Mean pneumonia lung lesion percentage for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA). Lung lesion % have been log_{10} + 0.1 transformed.

Treatment	Scan 1	Scan 2	Scan 3
1 – Control	-0.886[a]	-0.965[a]	-0.398[a]
2 – Pm	-0.804[a]	0.426[b]	0.29[b]
3 – Mhp	0.168[b]	0.778[c]	-0.417[a]
4 – Mhp+Pm	0.115[b]	1.098[d]	0.656[b]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

Two to three weeks after subsequent challenge with Pasteurella (scan 2), pigs challenged with both pathogens had the highest mean percentage of pneumonia relative to all other treatments, suggesting that the earlier Mhp induced lesions had not yet resolved. Interestingly, the percentage of pneumonia affected lungs continued to increase between scan 1 and 2 (2.5 and 5.5 weeks after initial Mhp challenge) in pigs challenged with Mhp alone, suggesting that pneumonia lesions induced by Mhp required more than 3 weeks to be detected by the CT scanner. Pigs challenged with Pasteurella alone had lower lung lesions scores than pigs challenged with both pathogens at scan 2, but not scan 3. Both Pm challenged groups had significantly higher mean lung lesions at Scan 3 than the other two treatments, with most lesions resolving in the Mhp only treatment (Table 12).

Table 13: Bartlett's test for significant variation in pig lung lesions (%) between treatments. Lung lesion % have been log_{10} + 0.1 transformed.

Treatment	Scan 1	Scan 2	Scan 3
1-Control	0.102[a]	0.019[a]	0.433[a]
2-Pm	0.181[a, c]	0.602[b]	0.636[a]
3-Mhp	0.445[b]	0.05[c]	0.323[a]
4-Mhp+Pm	0.315[c]	0.098[c]	0.181[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

The greatest variation in percentage pneumonia affected lung was seen in the Pm only challenged pigs at scan 2 relative to all other treatments (Table 13). While Mhp+Pm challenged pigs had the least variation at Scan 3, there was no significant difference in variation between treatments at this time.

5.1.4 Carcass composition – Muscle and fat

As there is no 'time zero' (i.e. before Mhp challenge) scan, it is not possible to draw valid conclusions as to the effect of Mhp on body composition. However, comparison of the two replicates of Mhp challenged pigs (3 and 4) with the control pig replicates (1 and 2) at Scan 1 can give some indication as to whether the disease has caused changes in body composition.

Table 14: Mean P2 fat and muscle depth (mm) for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	Scan 1		Scan 2		Scan 3	
	P2 Fat	P2 Mus	P2 Fat	P2 Mus	P2 Fat	P2 Mus
1 – Control	3.35[a]	30.31[a]	5.4[a]	39.84[a]	6.76[a]	44.48[a]
2 – Pm	3.91[a]	29.94[a]	4.96[a, b]	37.05[a]	6.8[a]	43.83[a]
3 – Mhp	4.09[a]	33.96[b]	5.43[a]	39.41[a]	6.36[a]	44.64[a]
4 – Mhp+Pm	3.45[a]	32.16[a, b]	3.97[b]	36.22[a]	4.94[b]	42.62[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

There was no significant difference in mean P2 fat depth 3 weeks after Mhp challenge (Scan 1), but there was a trend toward increased fat depth in one Mhp replicate (3) (P=0.128). There was a significant difference in P2 muscle at this time, with the Mhp replicate (3) having greater muscle depth

than the control pigs (1 and 2) (Table 14). Variation in P2 fat and muscle was not significantly different between treatments (± Mhp, results not shown), however the control replicate tended to have more variation (P=0.055).

Two to three weeks after Pasteurella challenge, Mhp+Pm pigs had significantly lower mean P2 fat depth relative to the Mhp only and control pigs, and this continued to 6 weeks post Pm challenge (Table 14, scan 2 and 3). While there was no significant difference in mean P2 muscle between treatments at Scan 2 and 3, there was a trend for Mhp+Pm pigs to have less muscle depth at Scan 2 (P=0.158) (Table 14).

Respiratory diseases had no effect on variation in P2 muscle depth in this trial. However, variation in P2 fat depth was significantly lower in both groups challenged with Pasteurella at 5 to 6 weeks post challenge (Scan 3, not shown). Control and Mhp pigs tended to be more variable in P2 fat depth at Scan 2 (P=0.128), while both Pm challenged groups tended to be more variable for P2 muscle at the same time (P=0.07).

Mycoplasma infection did not affect either the mean or variation in the percentage volume of fat or muscle (Table 15, scan 1). However, there was a trend for increased mean % fat in Mhp challenged pigs (group 3) relative to control pigs (group 1) (P = 0.077), but this wasn't supported across both replicates of infected and control pigs, suggesting that environmental factors may also be relevant. While there was no overall effect of treatment on variation, there was a trend for increased variation in % fat in one of the Mhp infected replicates compared to one of the control replicates. When both infected and control replicates were included, there was no effect of treatment on variation in %fat; that is variation was greatest between replicates within the same treatment.

Table 15: Mean percentage weight of fat and muscle for control pigs (1), pigs challenged with single infections of Pm (2) or Mhp (3) or pigs challenged with both pathogens (4) (ANOVA).

Treatment	Scan 1		Scan 2		Scan 3	
	% Fat	%Muscle	% Fat	%Muscle	% Fat	%Muscle
1 – Control	9.41[a]	64.88[a]	13.12[a]	62.63[a]	15.06[a]	61.27[a]
2 – Pm	10.58[a]	63.55[a]	12.69[a]	62.53[a]	15.09[a]	61.24[a]
3 – Mhp	11.03[a]	63.66[a]	12.68[a]	63.02[a]	14.38[a, b]	61.48[a]
4 – Mhp+Pm	10.11[a]	64.42[a]	10.24[b]	64.87[b]	12.25[b]	63.48[a]

[a,b] Different superscripts indicate significant difference between values in a column (P<0.05)

Two weeks after secondary challenge with Pasteurella, pigs challenged with multiple pathogens had significantly reduced % fat and increased % muscle relative to all others (Table 15, scan 2). Five to six weeks after Pm challenge (Scan 3), pigs infected with multiple pathogens showed a reduced fat percentage compared to Pm only and control pigs. There was also a trend for Mhp+Pm pigs to have greater mean muscle percentage at Scan 3 (P=0.13). There was no significant effect on fat or muscle variation after Pasteurella challenge (data not shown).

5.2 Literature Review: Factors that impact on the expression of respiratory disease and how to monitor them

5.2.1 Respiratory disease definitions

Many potential bacterial pathogens colonize the nasal cavity or tonsils of pigs, but the host's defence mechanisms prevent damage or spread to the lungs. Primary pathogens are able to avoid these defence mechanisms and infect the lungs. Secondary or opportunistic pathogens are found in healthy pigs and require some modulation or compromise to the immune system before they can establish infections. Although primary respiratory pathogens can cause serious disease on their own, many cause uncomplicated infections with mild and transient disease. However, when these primary infections become complicated with opportunistic bacteria the severity of clinical signs and pneumonic lesions increases. Primary agents in pigs include *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica,* porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine circovirus type 2 (PCV2). Secondary or opportunistic pathogens include *Pasteurella multocida Mycoplasma hyorhinis, Haemophilus parasuis, Streptococcus suis, Actinobacillus suis, Arcanobacterium pyogenes* and *Escherichia coli* (Van Alstine, 2012).

Respiratory diseases can affect the nasal turbinates of pigs causing atrophic rhinitis (*B.bronchiseptica* and *P.multocida*). Pathogens can also affect the lungs causing pneumonia, including *A.pleuropneumoniae, A.suis, M.hyopneumoniae, H.parasuis, S.suis, P.multocida, B.bronchiseptica*, SIV, PCV2 and PRRSV, though this last virus is not present in Australia. Many respiratory pathogens are ubiquitous in commercial production facilities, so infection with multiple infectious agents (both bacteria and viruses) is not uncommon. The term porcine respiratory disease complex (PRDC) is used to describe the disease caused by multiple pathogens, which is exacerbated by environmental, genetic and management factors (Brockmeier et al., 2001). This literature review will focus on the pathogens and diseases of major importance to Australia including the causes of enzootic pneumonia (*M.hyopneumoniae*) and pleuropneumoniae (*A.pleuropneumoniae*), with less emphasis on the causes of pneumonic pasteurellosis (*P.multocida*), and Glässers disease (*H.parasuis*).

Non-infectious factors also contribute to the expression of disease including the host's genetics and immune status and environmental and management factors including air quality, temperature, humidity, nutrition, herd size and production flows. All of these factors can be measured and, if correlated with production parameters, may prove helpful in monitoring respiratory disease expression and recovery in pig herds.

5.2.2 Why is respiratory disease important to the Australian pig industry?

The most prevalent respiratory diseases are pneumonia, pleuropneumonia and pleurisy, with high morbidity and variable mortality, depending on the causative agents. The economic impact of respiratory diseases are considerable, due mainly to reduced growth and feed efficiency and to the prevalence of disease (Straw et al., 1990). Significant economic losses are also due to increased medication use or other treatment costs. Enzootic pneumonia has been reported to impact on ADG, ADFI, feed efficiency and carcass composition in pigs naturally or experimentally challenged with *M.hyopneumoniae* (Eamens et al., 2007; Escobar et al., 2002; Maes et al., 1999; Maes et al., 1996; Morris et al., 1995a; Wyburn et al., 2015). Linear relationships between pneumonia severity and pig growth have been derived (Straw et al., 1989), although there is evidence that low levels of pneumonia do not greatly affect growth and that growth may be a poor indicator of pneumonia severity (Eamens et al., 2007; Eamens et al., 2011). Reductions in growth rate and feed efficiency of up to 26% and 20%

respectively have been reported (Zimmerman et al., 1973). Pigs naturally infected with *M.hyopneumoniae* as growers suffered reduced growth by 12.7%, but this was increased to 15.9% in pigs infected as weaner-growers with feed conversion reduced by 13.8% (Pointon et al., 1985). Another estimate of pneumonia's impact indicated that every 10% of lung affected with pneumonia reduced average daily gain by between 24.8 and 37.5g and feed efficiency by 4.5% or up to 13.4% in pigs with 50% of lung volume affected (Straw et al., 1990; Straw et al., 1989).

The cost of pneumonia in Australian herds was estimated to be A$2.80 per pig, where growth losses equated to 12 to 15% in grower/finisher pigs and 40% of lungs were affected with pneumonia at slaughter (Pointon et al., 1985). A British study found that the cost of pneumonia varied from £1 to £5.70 per pig when the extent of pneumonia increased from 5% to 20% of lung volumes (Burch, 2007). The cost of NZ $1 was estimated for every percent of lung volume affected with pneumonia (Christensen, 1995). The economic and production impacts of respiratory diseases are more severe if they occur early in life (Morris et al., 1995a; Sitjar et al., 1996) or are aggravated by other diseases or an adverse environment (Brockmeier et al., 2001; Donham, 1991; Hamilton et al., 1999; Pointon et al., 1985).

The prevalence of pneumonia lesions at Australian abattoirs has been reported to be as high as 47% (Pointon and Sloane, 1984), with one study showing extremely high within herd prevalence (greater than 70%) in up to 21% of herds (Pointon, 1990). World-wide, enzootic pneumonia has been estimated to be present in 96% of pig herds, and the prevalence in US herds has been estimated to be as high as 70% of pigs (Muller and Abbott, 1986).

Increasing pneumonia severity also led to increased numbers of days to slaughter and reduced live weight at slaughter (Christensen, 1995). *M.hyopneumoniae* infection did not affect whole body fat or protein accretion but did increase muscle depth at the P2 back position (Eamens et al., 2007; Escobar et al., 2002). However, when *M.hyopneumoniae* was combined with a secondary *P.multocida* infection, pigs showed a significantly reduced P2 back fat depth and total percent body fat and an increased percent muscle volume (Eamens et al., 2007).

Stimulation of the immune system by respiratory pathogens can impact on the pig's metabolism, as nutrients and energy are sequestered away from muscle deposition for the production of acute phase proteins, antibodies and cytotoxic and regulatory T cells to help fight infection (Black and Pluske, 2011). In particular it is the release of pro-inflammatory cytokines, causing fever, anorexia and lethargy, which impact on metabolism. *M.hyopneumoniae* infection alone (Woolley et al., 2012; Woolley et al., 2013) or a combined infection with PRRS resulted in the production of pro-inflammatory cytokines (IL-1ß, IL6 and TNFα), which are associated with increased severity and duration of pneumonia (Thacker et al., 2000). Pneumonia induced by *P.multocida* alone or combined with prior *M.hyopneumoniae* infection had adverse impacts on energy intake and retention, resulting in energy intakes of less than 25 MJ/d and energy retentions of less than 10 MJ/d during the acute phase of disease in grower pigs (Eamens, 2006).

Significant variation in pig production, particularly ADG, ADFI, gain:feed ratio and days to slaughter has been reported when *M.hyopneumoniae* infection occurs in combination with other bacterial and viral respiratory pathogens, exacerbating production losses relative to single infections (Collins and Wyburn, 2015; Opriessnig et al., 2011). Variation in production parameters due to pneumonia is perhaps not surprising considering the variation also observed in the volume of pneumonic lesions between pigs challenged with the same dose of *M.hyopneumoniae* and housed under the same

environmental conditions (Lorenzo et al., 2006; Sibila et al., 2007). Variation in the severity of pneumonic lesions suggests significant variation in the pigs' immune response to infection. As energy is required to raise an effective immune response, it is also likely that pneumonia causes variation in energy expenditure and the resultant pig growth (Bray et al., 1995). Variation in growth within batches of pigs can compromise all-in all-out (AIAO) production and extend time to slaughter. Variation in carcass composition can incur penalties at processing, reducing profit and limiting marketing options (Payne et al., 1999).

Although variation in growth and feed intake in pigs is acknowledged to be an obstacle to efficient production by increasing the proportion of 'tail-ender' pigs (Magowan et al., 2007; Patience et al., 2004; Payne et al., 1999), very little research has focused on the impact of chronic and acute disease on variation in production parameters. Variation in growth and feed intake will have significant effects on the length of time needed for a batch of pigs to reach slaughter weight, and the uniformity of carcass composition at processing (Collins and Wyburn, 2015).

Variation in market weight impacts on profitability by increasing housing costs per pig, due to delayed emptying of sheds for light weight pigs in AIAO production (Magowan et al., 2007). To avoid variation in market weight, producers often sort pigs on weight before sale. However, mixing pigs or changing to continuous flow production increases the risk of further outbreaks of pneumonia (grosse Beilage et al., 2009) and frequent mixing of pigs may increase fighting and stress (Maes et al., 2008). Marketing pigs at different slaughter weights to the most appropriate buyer will allow continuation of AIAO production, but can incur additional costs in sorting and transport.

Variation in carcass size, shape and composition will also affect profitability due to increased processing times for handling non-uniform carcasses and increased numbers of carcasses failing to meet the target grade (Patience et al., 2004; Tokach, 2004). Although increased variation in fat and muscle percentages or P2 muscle depth could not be demonstrated in pigs experimentally affected with pneumonia (Collins and Wyburn, 2015), it is possible that this could change under field conditions. Additional environmental stressors, such as repeated challenge, temperature fluctuations, ammonia and dust particles, are likely to exacerbate the immune response to pathogen challenge and could lead to more significant variation in production parameters and body composition (Stärk, 2000).

Vaccination, medication and eradication are the main tools used to control respiratory disease in pigs, and all incur significant costs to the producer. Eradication of *M.hyopneumoniae* has been undertaken extensively in Finland, Denmark and Switzerland using the "Swiss depopulation" method, where all animals under 10 months of age are removed from the herd. The remaining pigs are medicated with antibiotics and then production can recommence. This protocol has been modified in Canada and the US to include vaccination of the breeding herd. This is obviously a costly procedure in terms of time and money, so great care needs to be taken to prevent the re-introduction of respiratory pathogens into naïve herds. The distance to the closest farm, the size of the neighbouring farms and the pig density in the area are all important factors in the risk of re-infection post eradication of respiratory disease (Stark et al., 1992). In Finland, eradications are undertaken on a broad scale and all farrowing sows are tested for *M.hyopneumoniae* antibodies in colostrum to demonstrate the pneumonia-free status of the herd. The ELISA is more sensitive on colostral samples than on serum (Sorensen et al., 1993), but more than 30 colostrum samples are needed per herd to demonstrate freedom from disease (Rautiainen et al., 1996).

M.hyopneumoniae vaccination can improve pig performance; reduce clinical signs, lung lesions and the number of days to slaughter (Haesebrouck et al., 2004), but no vaccines are currently able to prevent colonisation by respiratory pathogens. Vaccination with *M.hyopneumoniae* does not lead to a humoral immune response in all vaccinated animals; the proportion of animals seroconverting varies from 30 to 100% (Maes et al., 2008; Sibila et al., 2004). Serum antibodies to *M.hyopneumoniae* do not demonstrate protection from pneumonia (Djordjevic et al., 1997). Vaccination also induces a cell mediated immune response, with elevated numbers of IFN-γ secreting cells, suggesting that IFN-γ may be important in protection against *M.hyopneumoniae* (Thacker et al., 2000). Vaccines can also reduce the prevalence of *M.hyopneumoniae* found in the nasal cavity and tonsils, and induce seroconversion in a greater proportion of pigs at an earlier time following secondary natural exposure (Sibila et al., 2007).

Vaccinating sows at the end of gestation will protect piglets due to the transfer of maternal antibodies in the sow's colostrum and milk (Sibila et al., 2006). However, maternal antibodies do not prevent *M.hyopneumoniae* colonization (Thacker, 2006). Pigs can be vaccinated pre-weaning, to prevent transmission from the sow, but maternal antibodies may interfere with the vaccine.

Demonstrating the efficacy of vaccines against respiratory disease is hampered by the presence of confounding disease factors. Other respiratory pathogens and environmental conditions can continue to cause clinical respiratory disease signs or production losses. The two most common ways to evaluate the efficacy of *M.hyopneumoniae* vaccines in real time are monitoring coughing scores and serology. If coughing scores or production losses don't improve following vaccination, then further investigations are needed to determine what other pathogens or conditions may be causing the observed respiratory disease.

Control of respiratory disease with antimicrobials is often effective because they can work against several pathogens and they are usually more cost effective. However, continued antimicrobial use can select for resistance in pathogenic bacteria and care needs to be taken to ensure that antimicrobial residues are not present in food animals. Antimicrobials also do not prevent attachment and infection by *M.hyopneumoniae*.

5.2.3 What parameters can be measured and do they correlate with expression of respiratory disease?

Respiratory disease prevalence and severity can be estimated using a wide range of parameters including clinical signs (fever, coughing, and dyspnea), lung lesions at slaughter, immune response (serum and colostral IgG) and pathogen detection in tissue or secretions of affected pigs. The non-specific nature of clinical signs associated with respiratory disease mean that it is quite difficult to measure the impact of specific respiratory diseases in real time. Coughing, fever, dyspnoea, lethargy and inappetance may be due to one of many respiratory pathogens (Table 16). While production measures such as feed intake and weight gain are related to the severity of pneumonia, they are not specific measures for respiratory pathogens. Lung lesions at slaughter are an accurate measure of pneumonia or pleurisy severity but only provide evidence of previous disease and therefore delay the implementation of disease controls. In the following section, the diagnostic assays developed to diagnose respiratory disease will be evaluated for their ability to measure disease expression.

Table 16: Clinical signs caused by respiratory pathogens of pigs

Pathogen	Clinical signs
H.parasuis	Fever, coughing, paddling, trembling, reduced growth, lameness and lateral recumbency, dyspnoea (laboured breathing)
M.hyopneumoniae	Fever*, dry, non-productive cough in finishers, decreased appetite*, lateral recumbency* and dyspnoea*
P.multocida	Sneezing and snout deformation
A.pleuropneumoniae	Fever, anorexia and apathy, coughing more common with chronic disease, dyspnoea
B.bronchiseptica	Coughing sneezing, nasal discharge, lethargy, dyspnea
Influenza A virus	Fever, anorexia, huddling, rapid breathing and coughing

* Occur with secondary infections

Coughing scores

Coughing scores focus on calculating the percentage of coughing outbreaks for groups of pigs within a short period after moving them (Nathues et al., 2012). A strong positive correlation between coughing and lung lesion score has been shown in experimental *M.hyopneumoniae* challenge trials (Kobisch et al., 1993). Gross lesions of pneumonia four weeks after experimental challenge with different field isolates of *M.hyopneumoniae* correlated strongly (Spearman's correlation coefficients > 0.8) with the number of coughing days, the severity of coughing and the intensity of immunofluorescence antibodies bound to *M.hyopneumoniae* in lung tissue (Vicca et al., 2003). However the sensitivity and specificity of coughing as an indicator of lung lesions in commercial herds at slaughter was only moderate (37.7% and 76.3% respectively). Coughing is not considered a specific clinical sign to identify the cause of respiratory disease (Morris et al., 1995a), although a dry, non-productive cough in finisher pigs is a strong indication of enzootic pneumonia. In experimentally infected pigs, chronic non-productive coughing occurred 10 to 16 days after *M.hyopneumoniae* challenge, but the timing of coughing is much more variable under field conditions. In field studies, coughing scores failed to detect 30% of infected herds (Sorensen et al., 1993) and are considered unlikely to detect sub-clinically affected herds (Keller, 1976). More recently software has been developed capable of automatically detecting coughing and of distinguishing between coughs from healthy pigs (throat cleaning to clear dust), and coughs by pigs infected with *Pasteurella multocida* and *A.pleuropneumoniae* (Giesert et al., 2011). However, this has yet to be evaluated extensively under field conditions.

While coughing is not considered to be a good indicator of respiratory disease when used on its own, it could prove useful in a combined respiratory health (RHS) score. (Hoeltig et al., 2009). An RHS score was developed for pigs infected with *A.pleuropneumoniae,* which combined lung lesions measures (sonographic and radiographic) with clinical scores. Clinical scores measured general appearance and behaviour of the pig (feed intake, temperature, vomiting and recumbency), as well as specific respiratory disease signs (breathing noise, dyspnoea, coughing and cyanosis). The ultrasound and radiography measured lung alterations in real time and scored tissue on the level of damage. Obviously these lung lesions measures are not practical in commercial herds.

Clinical signs (coughing) and lung lesions can provide a tentative diagnosis of enzootic pneumonia, but laboratory tests (pathogen or antibody detection) are required for a conclusive diagnosis at the herd level. Diagnosis of enzootic pneumonia from 59 German pig herds using coughing index was strengthened by including the *M.hyopneumoniae* PCR results from bronchoalveolar lavage (BAL) fluid and serology results using the blocking ELISA. If these herds were segregated into high (≥ 50% positive)

and low (≤ 50% positive) prevalence of *M.hyopneumoniae* based on serology and PCR, both pathogen or antibody detection were strongly associated with the coughing index in the same herd (Nathues et al., 2012). The authors suggested that enzootic pneumonia was highly likely in herds where the coughing index was above 2.5% and the seroprevalence was greater than 50%. Alternately, when more than 50% of pigs in a herd were seropositive, the risk of an increased coughing index was increased by 50%. The chance of a higher coughing index was increased by 76% in herds where more than 50% of BAL fluids were PCR positive (Nathues et al., 2012).

Gross lung pathology

Diagnosis of respiratory disease can also be based on gross pathological lung lesions at slaughter, with the volume of pneumonia lesions scored visually (Goodwin et al., 1969). A minimum of 30 lungs per batch of pigs needs to be examined at slaughter to reliably estimate the prevalence and severity of pneumonia at the herd level (Davies et al., 1995; Pointon et al., 1990). If relying on slaughter checks to diagnose enzootic pneumonia at the herd level, somewhere between 10% and 25% of pigs needed to be affected with gross lung pathology (Tielen et al. 1978, referenced by (Stärk, 2000)). However, pneumonic lesions at slaughter are not a good indicator of neither the lifetime impact of pneumonia, nor the peak in pneumonic lesions, nor the associated production effects (Noyes et al., 1990a; Sitjar et al., 1996). It has been estimated that only one-third of pneumonia lesions from early infection are still active at slaughter (Wallgren et al., 1994), and a large proportion will have completely resolved (Stärk, 2000).

The volume of pneumonia affected lung tissue can be measured in real time by computed tomography (CT), because the density of healthy and pneumonic lung tissue differs (Akira et al., 2000). Pneumonia lesion volumes (estimated by CT) were significantly higher in pigs challenged with *M.hyopneumoniae* or *P.multocida* or both pathogens relative to control pigs (Eamens et al., 2007). The volume of pneumonic lesions (visualised by CT) also correlates positively with clinical scores (coughing) and negatively with animal activity, i.e. sick animals were less active.

Diagnosis of respiratory disease is improved when serological, histology or microbiological assays are performed in combination with clinical coughing scores and lung pathology. A very strong correlation (0.94) exists between histological lesions of enzootic pneumonia and gross rapid examination at the abattoir. If histopathology was considered the gold standard in diagnosis, then gross pathology at slaughter had a sensitivity of 76% and a specificity of 71% (Hurnik et al., 1993). Consecutive monitoring of pigs every 2 weeks for weight gains, lung lesions (radiography) and antibodies against *M.hyopneumoniae* and *A.pleuropneumoniae* indicated that seroconversion to *M.hyopneumoniae* correlated significantly with the peak in pneumonic lesions, and average daily gain correlated negatively with the average lifetime pneumonia score (total of radiography scores). However, slaughter lesions did not correlate with the peak in pneumonic lesions or the average lifetime pneumonia score (Sitjar et al., 1996). Seroconversion coincides with the onset of coughing, but only moderate agreement between serology and gross lesions at abattoir have been reported (Morris et al., 1995a; Morris et al., 1995b).

Serology

Innate immune responses are the first line of attack against pathogens, including phagocytosis, the production of inflammatory cytokines and acute phase proteins. Acute phase proteins (APP) are induced by cytokines at the site of injury or infection, but are synthesized by the liver, and thus are detected in the circulatory system. Increased concentrations of APP are detected following surgery

(Jacobson et al., 2001), inflammation (Eckersall et al., 1996), immunization (Dritz et al., 1996) and infection (Heegaard et al., 1998). Cytokines and APPs are considered a good measure of infection because levels elevate rapidly and conversely they have a short half-life in serum. They are also detected in sub-clinically affected animals (Eurell et al., 1992). However, they are not a specific measure of disease, and high variation between individuals makes it difficult to interpret values. However, cytokines are a good measure of lung tissue damage following both bacterial and viral infections. Measuring pro-inflammatory cytokines and acute phase proteins will help determine a host's ability to resist a pathogen. Measuring antibody titres, pathogen loads and anti-inflammatory cytokines (the negative feedback mechanism required to dampen the inflammatory response) may equate more closely with the host's ability to recover from infection.

In field studies of pigs with respiratory disease, increased numbers of polymorphonuclear neutrophilic granulocytes (PMN) and increased concentrations of the antibacterial peptide PR-39 were found in bronchoalveolar lavage fluid (BALF) (Hennig-Pauka et al., 2007). The PMN cells and PR-39 peptide are both involved in killing bacteria. The authors suggested measuring proportions of PMN cells (> 8%) and PR-39 concentrations (> 2.5nM) in BALF, along with an elevated coughing score as good markers for respiratory disease in pigs. However, PR-39 is also involved in wound repair and later studies indicated that PR-39 was not a good marker for respiratory disease because PR-39 was found in all tissue samples of the upper respiratory tract in healthy and diseased pigs (Hennig-Pauka et al., 2012).

Several ELISA methods have been developed for the detection of antibodies to *M.hyopneumoniae* with antigen prepared from whole cell extracts or membrane bound proteins. However, most of these are not specific due to cross-reactivity with *M. flocculare* and *M. hyorhinis.* The two most commonly used commercial ELISAs are the indirect ELISA using a detergent solubilized antigen (Tween 20) (Ross 1999; Wallgren et al., 1996) and a blocking ELISA using a monoclonal antibody against an internal *M.hyopneumoniae* protein (Feld et al., 1992). Both assays are able to accurately identify samples that are negative for *M.hyopneumoniae* antibodies, but have poor sensitivity in only detecting 37% and 49% of positives (indirect ELISA and blocking ELISA respectively) from individual experimentally challenged pigs (Erlandson et al., 2005). The blocking ELISA demonstrated higher sensitivity in early infection compared with the indirect ELISA (Fano et al., 2012). Antigenic variability in *M.hyopneumoniae* surface proteins may explain the relatively low sensitivity of the indirect ELISA (Young and Ross, 1987). Both of the commercial ELISAs show poor sensitivity with vaccinated animals and in naturally exposed pigs in the first 21 days post infection, so a combination of molecular and serological assays may be needed to increase the sensitivity for the diagnosis of *M.hyopneumoniae* infection (Erlandson et al., 2005). The ELISAs can be used to diagnose both clinical and subclinical mycoplasmal pneumonia on a herd basis, but are not useful for diagnosis in individual animals (Sorensen et al., 1997). In field trials the blocking ELISA showed a sensitivity and specificity of the 93–100% and 85–96%, respectively (Sorensen et al., 1993).

ELISAs against *A.pleuropneumoniae* have been developed to detect antibodies against capsular polysaccharides, O-chain lipopolysaccharides or the ApxIV virulence toxin (Dreyfus et al., 2004; Gottschalk et al., 1997; Inzana and Fenwick, 2001). The capsular polysaccharide ELISA is not considered specific for *A.pleuropneumoniae;* where seroconversion in pleuropneumonia-free herds is reported to be due to cross-reactions with other bacterial antigens (Eamens et al., 2008, 2012). Numerous studies have compared the sensitivity and specificity of serological assays for *A.pleuropneumoniae* and have produced conflicting results, which may be explained by the different target antigens (Dubreuil et al., 2000; Eamens et al., 2008; Habrun et al., 1998; Opriessnig et al., 2013).

An ELISA using the N terminal part of the ApxIV toxin was demonstrated to be 100% specific and 93.8% sensitive on individual pigs experimentally infected with *A.pleuropneumoniae* (Dreyfus et al., 2004). While the ApxIV ELISA is highly specific and sensitive for all 15 serotypes of *A.pleuropneumoniae*, it can't differentiate between serotypes and therefore can't determine if new serotypes of *A.pleuropneumoniae* are responsible for disease outbreaks. In addition, the ApxIV ELISA can't detect herds infected with *A.pleuropneumoniae* strains that no longer express ApxIV toxin due to insertion sequences in the bacterial genome (Tegetmeyer et al., 2008). Serovar-specific ELISAs, like the LPS ELISA, are useful in studying the epidemiology of *A.pleuropneumoniae* in herds, where disease is associated with known serovars of *A.pleuropneumoniae* (Beynon et al., 1992).

Serum IgG antibodies to *A.pleuropneumoniae* can be used to diagnose pleuropneumonia at the herd level, with antibodies detected between two and four weeks after challenge (Marois et al., 2009) and two weeks after vaccination, depending on the adjuvant used (Hall et al., 1989). However, the delay in antibody production means that serology is often not useful for diagnosing pleuropneumonia in per-acute or acute cases, where pigs may die within hours or days. While antibodies may develop in surviving pigs, sub-clinically affected herds with carrier animals may appear negative on serology (Chiers et al., 2002). These sub-clinically affected pigs are a significant source of infection to in-contact pigs (Bosse et al., 2002). In a large slaughter survey, lesions of pleurisy did not correlate with either the timing of seroconversion or the antibody titre to *A.pleuropneumoniae* (Wallgren et al., 1994).

Antibodies to a range of pig respiratory pathogens can also be detected in oral fluids including *M.hyopneumoniae* (Gomes Neto et al., 2014), PCV2 (Prickett et al., 2011), *A.pleuropneumoniae* (Loftager et al., 1993) and Influenza A virus (Panyasing et al., 2014). *M.hyopneumoniae* IgA antibodies were detected in oral fluids earlier than serum IgG responses (Gomes Neto et al., 2014), so could prove useful as a monitoring tool. Mucosal IgA antibodies to *A.pleuropneumoniae* in oral fluids (saliva) and bronchoalveolar lavage fluid can be used to diagnose pleuropneumonia, and can be detected earlier in infection than serum IgG antibodies (Loftager et al., 1993).

Monitoring feed intake, weight gain and feed conversion ratio will certainly demonstrate disease severity and production losses, but they provide no information about the host's interaction with the causative pathogen. Measures of health, infection, immune stimulation, disease severity and recovery need to be correlated to feed intake and growth in order to quantify disease expression in pigs. Relationships need to be identified between immune responses and production parameters to increase the usefulness of immune markers to measure disease expression. Both adaptive and innate immune responses are necessary to help fight infection, but they also cause reduced appetite, fever and diversion of energy from growth to the immune system.

Culture or PCR detection of pathogens

Respiratory pathogens can be detected in the nasal cavities of grower pigs from nasal swabs (Goodwin, 1972; Kume et al., 1986; Willson et al., 1987; Sørensen et al., 1997) or tracheobronchial lavages (Abiven & Pommier, 1993; Ganter et al., 1993; Ganter, 1996) by culture or amplification by polymerase chain reaction (PCR). Culture is considered the 'gold standard' for diagnosis because of its sensitivity (Sorensen et al., 1997), but *M.hyopneumoniae* culture is relatively slow and *M.hyopneumoniae* has been cultured from lungs where no gross lesions were present (Goodwin, 1972). PCR is used frequently as a fast, sensitive and specific method for pathogen detection (Sirois et al., 1991; Mattson et al., 1995), but appropriate sampling and interpretation of results is critical. Detection of *Haemophilus parasuis, and Streptococcus suis* by PCR does not correlate with disease, as they are normal colonisers of the

upper respiratory tract and tonsils of healthy pigs (Amass et al., 1995; Smart et al., 1988). Likewise, the success in detecting *M.hyopneumoniae* by PCR or culture depends on the sampling site. Comparisons of sampling sites demonstrated that *M.hyopneumoniae* detection in trachea-bronchial swabbing or washing is more sensitive than nasal swabbing, tonsillar swabbing or oral-pharyngeal brushing (Fablet et al., 2010; Kurth et al., 2002; Marois et al., 2007). *M.hyopneumoniae* bacteria are primarily found on the mucosal surface of the trachea, bronchi and bronchioles (Blanchard et al., 1992). It is therefore not surprising that PCR detection in nasal swabs is less sensitive that bronchial swabs, and that pathogen numbers were 100 times higher in homogenized lungs compared to nasal swabs (Otagiri et al., 2005). The amplification of *M.hyopneumoniae* DNA from bronchial swabs by nested-PCR correlated well with histopathological lesions of enzootic pneumonia (Calsamiglia et al., 2000).The detection of *M.hyopneumoniae* in bronchoalveolar (BAL) fluid was also significantly associated with enzootic pneumonia lesions (Fablet et al., 2010; Sibila et al., 2007), but collection of BAL fluid requires anaesthesia of live animals.

Developing a quantitative PCR that is specific for *M. hyopneumoniae*, but also able to detect all *M. hyopneumoniae* isolates has proven difficult. The genetic variability between *M. hyopneumoniae* isolates means that PCR assays need to be validated with a large number of *M. hyopneumoniae* isolates, as well as against other *Mycoplasma* spp., including *M. flocculare, M. hyorhinis* and *M. synoviae* (Minion et al., 2000; Vicca et al., 2003). A specific and sensitive qPCR for *M. hyopneumoniae* (Mhp183 detecting the p97 adhesin) was developed (Strait et al., 2008) and validated with field and reference strains of *M.hyopneumoniae,* and other *Mycoplasma* spp. Another real-time triplex PCR developed to detect three adhesin proteins of *M.hyopneumoniae* (*p46, p97* and *p102* genes) indicated that the *p102* adhesin PCR was best able to detect all *M.hyopneumoniae* reference and field strains; whereas the *p97* PCR was unable to detect 18 field strains (Marois et al., 2010).

The advantage of the Mhp183 qPCR is that it was validated against histopathology on right and left lung samples from pigs challenged with 2 different *M.hyopneumoniae* isolates (Woolley et al., 2012). The Mhp183 qPCR was able to detect *M.hyopneumoniae* in the lung, spleen, liver and kidney tissues of *M.hyopneumoniae* challenged pigs. However, no significant correlations were observed between lung lesion severity (% pneumonic lung) and *M.hyopneumoniae* DNA concentrations in TBLF (Woolley et al., 2012). The Mhp183 qPCR was also able to detect *M.hyopneumoniae* in bronchial alveolar lavage fluid, nasal swabs and bronchial swabs of *M.hyopneumoniae* challenged pigs, but not control pigs (Strait et al., 2008). PCR detection from nasal swabs has been previously shown to be less sensitive than with bronchial swabs or bronchial alveolar lavage fluid, but this may depend on the *M.hyopneumoniae* challenge dose. When inoculated with 10^9 *M.hyopneumoniae*, only 7/10 nasal cavity swabs were positive by *p102* PRC, whereas 10/10 tonsil and tracheal swabs were positive (Marois et al., 2010). A more detailed study of the best sampling technique for *M.hyopneumoniae* detection by *p102* PCR indicated that tracheo-bronchial swabbing and trachea-bronchial washing were the most sensitive, and nasal swabs were the least sensitive in naturally infected finisher pigs (Fablet et al., 2010).

A.pleuropneumoniae can be isolated by traditional culture methods from freshly dead animals, but is rarely isolated from chronic cases of pleuropneumoniae or from lungs at the abattoir. A number of PCR assays have been developed to detect *A.pleuropneumoniae* in nasal and tonsillar swabs, oral fluids and affected lung tissue. PCR assays have either targeted the exotoxin *apx* genes or the outer membrane protein *omlA* gene. While all four *apx* exotoxin genes are used by *Actinobacillus* species for virulence, only the *apxIVA* gene is species-specific for *A.pleuropneumoniae* and is conserved across all serotypes (Schaller et al., 1999). Both nested PCR assays and real time PCR assays for the *apxIVA* gene

have proved as specific and at least as sensitive as bacterial culture from tonsillar swabs and affected lung tissue (Cho and Chae, 2001; Fittipaldi et al., 2003; Schaller et al., 2001; Tobias et al., 2012). *ApxIVA* is associated with pathogenicity of *A.pleuropneumoniae* in pigs, based on clinical signs and histopathology (Liu et al., 2009). Tonsillar swabs appear to be more sensitive than nasal swabs for quantitative PCR detection of *A.pleuropneumoniae* (Tobias et al., 2012).

A real time PCR for apxIVA was used to quantify the bacterial load of *A.pleuropneumoniae* in caesarean-derived, colostrum-deprived pigs experimentally challenged pigs, or in-contact pigs (Tobias et al., 2013). *A.pleuropneumoniae* numbers in tonsillar and nasal swabs were positively associated with disease score (behaviour, movement, vomiting, body temperature, respiratory sounds or dyspnoea and coughing) in inoculated pigs. The rate of transmission of infection from inoculated to in-contact pigs was also correlated with *A.pleuropneumoniae* numbers in respiratory tract swabs. However, there was a negative association between disease score and transmission rate i.e. in pigs shedding equal numbers of *A.pleuropneumoniae*, transmission rates to in-contact pigs were lower in pigs with higher disease scores, suggesting that shedding of *A.pleuropneumoniae* peaks before clinical disease is apparent. Good agreement ($\kappa = 0.68$) was found between serological status and the presence of *A. pleuropneumoniae* in the tonsils, using a conventional PCR for the *omlA* gene (Fittipaldi et al., 2003).

The respiratory pathogens *A.pleuropneumoniae,* influenza A virus, *H.parasuis* and *Streptococcus suis* can all be detected by PCR in oral fluids (Costa et al., 2012; Goodell et al., 2013). However the success and sensitivity of PCR assays depends on the bacteria being present in oral fluids, when their normal habitat may be in the lower respiratory tract. *M.hyopneumoniae* is yet to be reliably detected in oral fluids, possibly because mucin interferes with detection of the bacteria or also because *M.hyopneumoniae* mainly colonizes the lungs. Previous studies have reported that the optimal sample for PCR detection of *M.hyopneumoniae* is trachea-bronchial swabs or washings (bronchial alveolar fluid) (Fablet et al., 2010; Marois et al., 2010). It is therefore not surprising that the sensitivity of *M.hyopneumoniae* detection in oral fluids is fairly poor.

Although *A.pleuropneumoniae* colonizes the tonsillar crypts and would be expected to be present in oral fluids, the sensitivity of *A.pleuropneumoniae* detection in oral fluids is also poor relative to tonsillar swabs (Costa et al., 2012). *A.pleuropneumoniae* can be detected in oral fluids from pigs challenged with 10^6 virulent *A.pleuropneumoniae* on days 1 and 7 post challenge, but the detection sensitivity is relatively low compared to detection of the commensal bacteria *H.parasuis* and *S.suis* in the same samples (Costa et al., 2012). *Haemophilus parasuis, and Streptococcus suis* are normal colonisers of the upper respiratory tract and tonsils of healthy pigs (Amass et al., 1995; Smart et al., 1988) and can therefore be readily and frequently detected by PCR in oral fluids. Interestingly, antibodies to *A.pleuropneumoniae* were detected in oral fluids before serum, indicating that oral fluid testing could be useful for monitoring herds over time and specifically for biosecurity checks before introducing new stock (Loftager et al., 1993).

Oral fluid is an easier and less labour intensive sample to collect than nasal swabs or bronchoalveolar fluid. Detection of influenza A virus (IAV) by Real Time PCR from oral fluids provided the highest probability of detecting influenza A virus in pigs, although IAV replicates in bronchial epithelial cells (Goodell et al., 2013). As viruses are able to potentiate secondary bacterial infections, it may be worth monitoring influenza A virus loads in oral fluids to determine if this could be a predictor of risk for secondary infections or even a predictor of elevated respiratory disease expression.

Molecular techniques have also been used to rapidly serotype or genotype *A.pleuropneumoniae* strains by PCR (Bossé et al., 2014; Gram et al., 2000; Hennessy et al., 1993) or multilocus enzyme electrophoresis (Møller et al., 1992). Gram et al. (2000) developed a PCR system for typing the serovars of *A. pleuropneumoniae* based on the *omlA* and *apx* genes, which encode an outer membrane protein and exotoxin respectively. Serotypes could be distinguished using the *omlA*/*apx* PCR typing, with the exception of serotypes 1, 9, 11 and some field isolates of serotype 8. Serotype differentiation of *A.pleuropneumoniae* may be important in disease severity, but is more commonly used for epidemiology studies where the source of infection needs to be traced.

Pathogen load is believed to be a good indicator of disease severity and recovery from disease because pathogen load correlates well with disease measures in both enteric and respiratory infections. The severity of atrophic rhinitis correlates with the numbers of the respiratory pathogen *Pasteurella multocida* isolated from tonsils and nasal membranes (Hamilton et al., 1999; Hamilton et al., 1996). Quantitative PCR assays have been used to measure pathogen load in a wide range of clinical samples, both ante-mortem and post-mortem. The amount of porcine circovirus (PCV2) nucleic acids detected in tissues and serum is predictive of clinical outcomes (Opriessnig et al., 2007), which is critical with a pathogen that is found in both sick and healthy pigs. Numbers of more than 10^7 PCV2 genome copies per millilitre of serum differentiate PCV2 infection from PCV2 associated disease (Olvera et al., 2004; Brunborg et al., 2004). Where weight gain correlates well with specific pathogen numbers, quantifying the pathogen load over time can provide a measure of disease expression. This is more difficult to interpret when multiple pathogens or environmental conditions can exacerbate disease. A simple dose response relationship is rarely observed between pathogen numbers and disease because other factors in commercial herds also impact on the expression of respiratory disease. In addition, host factors appear to impact on the expression of respiratory disease because pigs challenged with the same dose of pathogen vary in their response. Without understanding the multifactorial causes of respiratory disease expression on farm, it's difficult for producers and veterinarians to prevent or treat disease.

5.2.4 Virulence factors can exacerbate respiratory disease expression

Pathogen virulence factors can affect the host's response to pathogen challenge and the associated expression of disease. A number of virulence factors have been suggested for *Actinobacillus pleuropneumoniae*, including capsular polysaccharides, lipopolysaccharides (LPS), surface proteins and extracellular toxins (Dubreuil et al., 2000), however the haemolysins are considered the main virulence determinants (Inzana, 1991). *A.pleuropneumoniae* serovars differ with respect to which haemolytic toxins they produce, but serotypes producing the haemolysin toxins ApxI and ApxII are considered the most virulent (Frey, 1995). Mutations of the haemolysin gene demonstrated that ApxI and ApxII are important in the pathogenicity of *A. pleuropneumoniae* and for virulence in mice and pigs (Tascon et al., 1994). Toxin ApxIVA is also associated with pathogenicity based on clinical signs and histopathology (Liu et al., 2009). Apx toxins are important in evasion of the immune system and can form pores in the membranes of phagocytes and other cell types causing cell death and stimulating the release of inflammatory mediators from activated phagocytes (Bosse et al., 2002). The combination of the cytotoxic effects of apx with the inflammatory response induced by LPS causes significant damage to lung tissue in *A.pleuropneumoniae* infected pigs (Frey, 2003).

The capsule protects *A.pleuropneumoniae* against phagocytosis and lysis by complement and is required for virulence of the bacterium. However, antibodies against the capsule alone are not enough to provide protection against *A.pleuropneumoniae* as non-capsulated mutants are protective while non-haemolytic and capsulated mutants aren't protective (Inzana, 1991). Lipopolysaccharide from

A.pleuropneumoniae has the potential to cause lung tissue damage, but does not induce typical haemorrhagic and necrotic lesions. Pigs immunized with LPS are only partially protected against challenge with the homologous *A. pleuropneumoniae* serotype (Inzana et al., 1988). However LPS may be important for pathogen adhesion (Paradis et al., 1994).

M.hyopneumoniae induces pneumonia pigs by damaging the ciliated epithelium. Adherence of *M.hyopneumoniae* to cilia lining the trachea of the swine respiratory tract leads to ciliostasis and loss of cilia (DeBey and Ross, 1994), and the infiltration of macrophages and lymphocytes to the lungs via the inflammatory cytokines IL-1, TNF-α and IL-6 (Choi et al., 1999). An outer membrane protein of *M.hyopneumoniae,* the P97 adhesin, mediates the adherence of *M.hyopneumoniae* to swine cilia (Minion et al., 2000). The P97/P102 paralog family of adhesins interact with porcine plasminogen and enhance its activation to plasmin (Seymour et al., 2012). Once plasmin is activated, it stimulates macrophage signalling leading to the release of cytokines (Syrovets et al., 2012). Pigs vaccinated against *M.hyopneumoniae* had reduced plasmin activity, as well as reduced clinical signs, pneumonic lesions, cytokines (TNF-α , IL-1β and IL-6) and *M.hyopneumoniae* numbers in tracheobronchial lung fluid (Woolley et al., 2013). A significant correlation was also observed between *M.hyopneumoniae* numbers and plasmin activity, indicating that plasmin activity enhances *M.hyopneumoniae* infection and therefore the tissue damage caused by the elevated inflammatory response.

Virulence factors in *M.hyopneumoniae* may relate to differences in genetics or antigenicity between isolates (Artiushin and Minion, 1996; Ro and Ross, 1983; Vicca et al., 2003). The cilium adhesin protein P97 has a region of 5 amino acids, termed R1, which when repeated in tandem at least 8 times allow for cilium binding to occur (Minion et al., 2000). Virulent strains of *M.hyopneumoniae* have higher numbers of repeats than avirulent strains.

Haemophilus parasuis, is both a common inhabitant of the upper respiratory tract of healthy pigs, but also the cause of Glässers disease, characterized by a fibrinous polyserositis and arthritis. *H.parasuis* strains differ in their virulence, and virulence appears to be correlated with the serotype of the strain (Nielsen, 1993). However, virulence may also be determined by the genotypes of isolates. Genetic typing of isolates from healthy and Glässers affected herds demonstrated that isolates that cause systemic disease are genetically separate from other isolates (Olvera et al., 2006; Smart et al., 1988). Systemic infection by *H.parasuis* requires strains to survive the host's immune defences in the lungs. *In-vitro* studies demonstrated that virulent strains of *H.parasuis* are resistant to phagocytosis by porcine alveolar macrophages (Olvera et al., 2009). Furthermore, virulent strains differed in their growth characteristics and localization in the respiratory tract. Virulent strains grew in biofilms in nasal turbinates and trachea and were also detected readily in lungs, whereas non-virulent strains were rarely detected in nasal turbinates and trachea, and almost never detected in lungs (Bello-Orti et al., 2014). Virulent strains were also sometimes found in association with macrophages and neutrophils.

5.2.5 Management factors that impact on respiratory disease expression

The impact of environmental and management factors on respiratory disease in pigs has been well reviewed (Maes et al., 2008; Stärk, 2000). Biosecurity, hygiene and all-in-all-out production are probably the most important factors in reducing the infection pressure or transmission of respiratory pathogens. Once the pathogen is present in the environment, the pig's susceptibility to infection is influenced by environmental factors (air quality, temperature, humidity, dust, gases and airborne bacteria) and host factors (genetics, immunity and other diseases) (Stärk, 2000).

Hygiene and Biosecurity

Internal biosecurity relies on practices such as all-in-all-out production (AIAO), which houses batches of the same age pigs together, thereby reducing the transmission of respiratory pathogens from older pigs to younger pigs (Scheidt et al., 1995). This practice helps to ensure a common immune status and pathogen exposure within a group of pigs. Complete AIAO production is difficult to accomplish in reality because disease outbreaks can cause variation in pig's growth, producing more 'tail-ender' pigs and therefore extending the days to slaughter for the batch. The loss in production days can be overcome if AIAO production is breached or if pigs are sold prior to optimal slaughter weights. To be effective, AIAO production also requires proper cleaning and disinfection of pens and sheds between batches to reduce the starting load of pathogens when new pigs are introduced.

Closing the herd to the entry of new animals can also reduce the risk of disease transmission. The risk of respiratory disease in more than 10% of animals was increased by 2.38 times when farms introduced new animals from herds without checking the health status of the source farm (Hurnik et al., 1994). Conversely, if new stock are introduced from a high health status herd, the odds ratio for *A.pleuropneumoniae* infection is less than 1, no matter whether one or 100 new animals are introduced (Rosendal and Mitchell, 1983). However, if care isn't taken regarding the health status of the source herd, the odds ratio of *A.pleuropneumoniae* infection increases with increasing numbers of animals; for 10 to 100 animals the odds ratio increases to 4.10, and for more than 100 animals the odds ratio increases to 6.89 (Rosendal and Mitchell, 1983). If new gilts are to be introduced, they should come from herds with a similar or higher health status and be housed in quarantine facilities for 30 days (Amass and Baysinger, 2006). The importance of strict biosecurity measures including insect and rodent control and restricted movement of personnel and equipment between animals of different ages has not yet been clearly demonstrated.

Early weaning of pigs (less than 3 weeks age) can reduce transmission of *M.hyopneumoniae* from the sow to her offspring, but there are welfare and production considerations associated with this practice (Maes et al., 2008). Transmission of *M.hyopneumoniae* can also be reduced by segregating gilt and sow progeny and gilts from sows until their second gestation, when gilts are expected to have developed immunity to *M.hyopneumoniae* (Maes et al., 2008).

The role of hygiene is particularly important in multifactorial respiratory diseases, where the environment also has a major impact on disease severity (Madec 2005). Under these circumstances hygiene practices need to reduce pathogen load, but also have to reduce environmental factors that exacerbate disease expression including production of excessive ammonia in the environment, dust and other pathogens. The health benefits of improved hygiene can rarely be attributed to a single factor. Stocking rate, herd size, air volume and quality, continuous production, temperature and humidity have all been identified as risk factors for respiratory disease in experimental challenge studies and epidemiological surveys (Pointon *et al.* 1985; Straw *et al.* 1991; Maes *et al.* 2000; Stärk 2000). Reducing stocking density also reduces the level of respiratory disease (Pointon et al., 1985). Guidelines for finisher pigs recommend a flooring space of at least 0.7m^2 per pig when housed on fully slatted floors. Higher densities increase the risk of pathogen spread and increase stress in pigs.

Environmental factors

In commercial pig herds, environmental factors that impact on the expression of respiratory disease include humidity, dust, ammonia, carbon dioxide, endotoxin and temperature. Elevated concentrations of ammonia and hydrogen sulphide gases and particulate materials like dust, skin and airborne bacteria

correlate with increased pneumonia and pleuritic severity and negatively affect the health and growth of pigs (Cargill and Skirrow, 1997; Donham, 1991; Robertson et al., 1990). Growth rates were reduced by 12% or 30% in growing pigs when ammonia levels increased to 50ppm and 100ppm (Drummond et al., 1980). In the case of atrophic rhinitis, both organic dust and atmospheric ammonia increased the severity of disease in pigs challenged with *P.multocida*, but some level of turbinate atrophy was detected in response to dust and ammonia alone (Hamilton et al., 1999). Dust, endotoxin, peptidoglycan from bacterial walls, ammonia and carbon dioxide in the airborne environment of pig sheds can induce immune cell activation and growth suppression in the absence of bacterial pathogens.

While Bordetella infection significantly reduces body weight in challenged pigs, the severity of atrophic rhinitis was related to the concentration of ammonia that pigs were exposed to (Drummond et al., 1981). Ammonia can also reduce the clearance of bacteria in the respiratory tract following aerosol exposure to non-pathogenic strains of *E.coli* (Drummond et al., 1978). The activity of cilia was also reduced in the presence of elevated levels of ammonia; potentially enabling the increased attachment and invasion of respiratory pathogens (Stärk, 2000).

Monitoring air quality may provide a better indicator of general pig health and growth than monitoring individual pathogen loads, where studies show that pigs housed in sheds with good air quality grow faster and consume more feed than pigs in sheds with elevated ammonia, carbon dioxide and dust levels (Lee et al., 2005). Concentrations of ammonia, carbon dioxide, inhalable dust, bacteria and endotoxin can all be quantified in livestock sheds and used to measure the impact of the environment on pig production and non-pathogen associated immune stimulation.

Air Quality

For respiratory disease, the available air quantity and quality, as well as floor space, impact on disease risks associated with stocking density. Recommendations of more than $3m^3$ air space per pig were developed based on the incidence of respiratory lesions at slaughter (Lindquist *et al.* 1974). Optimising ventilation to maintain air quality and air flow rates will also help to manage temperature and pathogen load (Eisenmenger, 2006). The relative risk of respiratory disease was increased by 1.55 times in the absence of a ventilation shaft in pig sheds (Aalund et al., 1976). An air exchange rate of more than 60 m^3 per hour per pig was reported to protect pigs form pneumonia (Flesja et al., 1982). The total and respirable dust levels in piggeries can be measured with personal gravimetric samplers, and ammonia and other gases can be measured with standard gas tubes (Cargill *et al.* 1997). When dust and ammonia levels increase, ventilation can be used to improve air quality. The load of bacteria in aerosols can be monitored by either traditional culture techniques or by the recently developed quantitative polymerase chain reaction (qPCR) assays that can also detect non-cultivable bacteria.

Monitoring respiratory pathogens in piggery air samples has only rarely been reported due to the perceived technical difficulty. Nucleic acids from *M.hyopneumoniae* can be detected by PCR in air samples from both experimental and commercial herds, and could prove a useful tool for measuring the risk of pneumonia transmission. *M.hyopneumoniae* DNA was detected in 53.6% of air samples from herds with acute respiratory problems, but was not detected from SPF herds that were sub-clinically affected (*M.hyopneumoniae* was present, but no clinical evidence of pneumonia) (Stärk et al., 1998). However, positive PCR results weren't clearly associated with pens where coughing pigs were observed. Recent technology has allowed for the quantification of respiratory pathogens in piggery air samples, which may prove useful to predict disease outbreaks. Combining real time PCR detection of pathogen numbers with commercial air samplers allows producers to estimate pathogen loads in set

volumes of air and to help identify practices that either exacerbate or reduce pathogen loads. Using this technology, increasing stocking density and increasing age of weaner pigs were both associated with increasing pathogen loads in piggeries (Marenda et al., 2014).

Aerial disinfection by fogging has been reported to reduce loads of both bacterial and viral respiratory pathogens in sheds while pigs are still resident (Fotheringham *et al.* 1995). More recently, electrostatic particle ionization sanitation of air in pig sheds has been trialled to help reduce the load of swine influenza virus and porcine reproductive and respiratory syndrome (PRRS) virus (Alonso *et al*. 2014a; 2014b).

Draughts in pens of weaner pigs reduced ADG, and increased sneezing, coughing and diarrhoea in herds with evidence of endemic pleuropneumonia, Aujeszky's disease virus and swine influenza (Scheepens *et al.* 1991). Cold air draughts and fluctuating temperatures influenced the immune system of pigs challenged with *A.pleuropneumoniae* (Noyes et al., 1990b). Increased pig activity levels associated with draughts led to increased dust levels and therefore increased the risk of aerosol transmission of respiratory pathogens (Scheepens, 1996). Controlling temperatures to be in thermal comfort zones will assist with reduction of stressors that predispose pigs to disease. External weather conditions can also impact on the risk of pleurisy and pneumonia, with more clinical disease expected in the colder, wetter months (Christensen, 1981; Stärk et al., 1992)

5.2.6 Host factors that impact on disease expression

Immune response

Immune competence is the ability of the host to raise an appropriate and effective immune response when exposed to an antigen (Wilkie and Mallard, 1998). Variation in the host's ability to resist disease is a combination of genetic factors and the health and functional state of its immune system (immune responsiveness). An animal's ability to exert control over a pathogen or resist infection can be measured by its immune responses (both innate and adaptive), but also by pathogen virulence factors and competitive inhibition by other bacterial colonisers at the site of infection. Measuring pro-inflammatory cytokines, acute phase proteins and ratios of commensal to pathogenic bacteria will help determine a host's ability to resist a pathogen. Measuring antibody titres, pathogen loads and anti-inflammatory cytokines (the negative feedback mechanism required to dampen the inflammatory response) may equate more closely with the host's ability to recover from infection.

Innate immune responses are the first line of attack against pathogens, including phagocytosis, the production of inflammatory cytokines and acute phase proteins. Acute phase proteins (APP) are induced by cytokines at the site of injury or infection, but are synthesized by the liver, and thus are detected in the circulatory system. Acute phase proteins can destroy or inhibit the growth of pathogens by a number of mechanisms, including opsonisation, recruitment of immune cells, induction of enzymes that degrade extracellular matrix, pathways of complement activation, coagulation of blood to trap pathogens in clots and a number of pathways to prevent iron uptake by microbes.

Cytokines may be a good measure of lung tissue damage following both bacterial and viral infections; however both acute phase proteins and cytokines are highly reactive to infection. The inflammatory cytokines IL-1, TNF-α and IL-6 are believed to play an important role in the early stages of *M.hyopneumoniae* infection. Elevated expression of these cytokines occurs in lungs as early as 7 days post *M.hyopneumoniae* challenge, when *M.hyopneumoniae* adhere to epithelial cilia. These cytokines regulate and mediate inflammatory cells, with increasing numbers of inflammatory cells (cytokine

positive) starting at 7 days post infection (pi) and increasing until 21 days pi, before decreasing (Choi et al., 2006). *M.hyopneumoniae* infection also leads to significantly higher expression of IL-2, IL-4, IL-8, IL-10 and TNF-α in the bronchus-associated lymphoid tissue of pneumonic pigs (Lorenzo et al., 2006). Elevated expression of these same cytokines, as well as IL-1α and IL-1β , were detected in the alveolar septa and bronchoalveolar exudate of these pneumonia-affected pigs. Elevated concentrations of IL-1β and IL-6 in tracheobronchial lavage fluid at 18 days post infection coincided with the peak in coughing scores (Woolley et al., 2012). While these cytokines help eliminate invading pathogens, overproduction of cytokines can lead to host-mediated tissue damage in the lungs.

Mucosal IgA antibodies to *M.hyopneumoniae* are able to opsonise the bacteria in the first sign of the humoral defence response. The production of circulating IgG antibodies in serum follows, detectable from two to four weeks post infection. Interestingly, IgA antibodies are detected in oral fluids earlier than serum IgG responses (Gomes Neto et al., 2014). Antibody production is a clear sign that the immune system is fighting infection and pneumonic lesions will start to recover, in the absence of secondary pathogens.

Any compromise to the pig's ability to raise both innate and humoral immune responses to respiratory pathogens will exacerbate disease. The clumping and loss of cilia, epithelial cell death and damage to the mucociliary apparatus following *M.hyopneumoniae* adherence is believed to increase the host's susceptibility to colonization by other bacterial and viral pathogens (DeBey and Ross, 1994). *M.hyopneumoniae* can also increase the host's susceptibility to secondary infections by suppressing the phagocytic activity of pulmonary alveolar macrophages and polymorphonuclear neutrophilic cells (PMN) (Asai et al., 1996; Caruso and Ross, 1990). A reduction in phagocytic activity of alveolar macrophages reduces the host's ability to clear *A.pleuropneumoniae* prior to the establishment of infection by reducing the concentration of complement and anti-*A.pleuropneumoniae* antibodies in the circulatory system (Cho et al., 2005).

The serum and tissue concentrations of the pro-inflammatory cytokines IL-1, TNF-α and IL-6 rise rapidly in the first 24 to 48 hours post infection with *A.pleuropneumoniae* (Baarsch et al., 2000). These cytokines mediate the infiltration of inflammatory cells into the lungs, and their expression is associated with pleuropneumonic lung lesions (Choi et al., 1999). *A.pleuropneumoniae* infection also leads to upregulated expression of acute phase protein genes in the liver and IL-1, IL-6 and IL-8 in inflamed lung tissue, along with LPS-binding protein (Hedegaard et al., 2007; Skovgaard et al., 2010). *A.pleuropneumoniae* infection also affects the pig's immune response by degrading extracellular matrix proteins and modulating the activity of cytokines that traffic inflammatory cells to airways (Bruun et al., 2012).

Serum IgG antibodies to *A.pleuropneumoniae* are detected between two and four weeks after challenge (Marois et al., 2009), so only develop in pigs that survive acute disease, in sub-clinically affected pigs and in carrier animals (Chiers et al., 2002). Mucosal IgA antibodies to *A.pleuropneumoniae* can be detected in oral fluids (saliva) and bronchoalveolar lavage fluid earlier than serum IgG antibodies(Loftager et al., 1993). Antibody production is important in recovery from disease, but lesions of pleurisy do not correlate with either the timing of seroconversion or the antibody titre to *A.pleuropneumoniae* (Wallgren et al., 1994) in slaughter surveys.

Haemophilus parasuis infection also leads to early innate immune responses including toll-like receptors, complement and coagulation pathways (Wang et al., 2012a) (Wang et al., 2012b). Pro-inflammatory

cytokine genes (especially IL-1β) were upregulated in both the lungs and porcine alveolar macrophages from bronchoalveolar lavage of Glässers susceptible pigs (Wang et al., 2012b; Wilkinson et al., 2010). Pigs more susceptible to *H.parasuis* challenge also showed an imbalance in the signalling between pro and anti-inflammatory cytokines. Pigs able to resist *H.parasuis* infection showed an increased expression of IgA; whereas susceptible pigs showed a reduced expression of antigen presenting genes (MHC I and II) by 72 hours post infection.

Other pathogens present in the host

Porcine respiratory disease complex (PRDC) describes pneumonia caused by multiple infections leading to poor weight gain, particularly in the later finishing period (15 to 20 weeks of age). PRDC is a multifactorial respiratory syndrome that includes several respiratory pathogens (bacterial and viral), that cause more severe clinical signs and disease when acting in concert than when acting alone. Environmental conditions, management factors, pig genetics and age also play a critical role in PRDC. Generally primary pathogens are viruses or mycoplasmas, with bacteria commonly infecting as secondary pathogens. Primary pathogens can predispose the pig to secondary pathogens by suppressing the local or systemic immune response. The adherence of *M.hyopneumoniae* to ciliated respiratory epithelium results in clumping and loss of cilia, epithelial cell death and damage to the mucociliary apparatus (DeBey and Ross, 1994). This damage is believed to increase the host's susceptibility to colonisation by other bacterial and viral pathogens. Pigs infected with *M.hyopneumoniae* then challenged with *Pasteurella multocida* suffered more severe clinical signs (fever, coughing, lethargy), significantly more extensive pneumonia lesions, altered body composition and reductions in weight gain, feed intake and live weight at 20 weeks of age (Amass et al., 1994; Ciprian et al., 1988; Eamens et al., 2007). Damage to the respiratory tract by *M.hyopneumoniae* also increased the severity of clinical signs, lung lesions and reduced average daily gains when coupled with a secondary *A.pleuropneumoniae* challenge (Ciprian et al., 1994; Marois et al., 2009).

Prior infection with *M.hyopneumoniae* can also exacerbate subsequent viral infections with PCV2 (Opriessnig et al., 2004), PRRS (Thacker et al., 1999; Thanawongnuwech et al., 2004; Thanawongnuwech et al., 2001), Swine Influenza Virus (SIV) (Thacker et al., 2001) and pseudorabies virus (PRV) (Shibata et al., 1998). Prior *M.hyopneumoniae* infection increased viral loads and the severity of pneumonia lesions. It has been suggested that the increased inflammatory response to multiple pathogens (induced by pro-inflammatory cytokines) may contribute to the severe chronic clinical signs and lesions of pneumonia seen in pigs co-infected with *M.hyopneumoniae* and PRRSV (Thanawongnuwech et al., 2004; Thanawongnuwech et al., 2001).

As a primary pathogen, the PRRS virus has multiple effects on the pig's immune response that make the host more susceptible to secondary infection. The PRRS virus invades and replicates in pulmonary macrophages, rapidly reducing their viability and phagocytic activity (Chiou et al., 2000), then induces their lysis (Thanawongnuwech et al., 1997), but also induces apoptosis in macrophages not infected with PRRSV (Labarque et al., 2003). The destruction and decreased function of pulmonary macrophages compromises the immune system, which is believed to result in increased susceptibility to bacterial pathogens (Brockmeier et al., 2002). Experimental co-infections of PRRS and *B.bronchiseptica* caused increased pulmonary lesions (Brockmeier et al., 2000). However, experimental co-infection of PRRSV with *P.multocida* or *M.hyopneumoniae* failed to increase the severity of clinical signs associated with the secondary pathogens (Brockmeier et al., 2001; Carvalho et al., 1997; Thacker et al., 1999). Primary infection with both PRRSV and *B.bronchiseptica* did predispose pigs secondary infections with *P.multocida* (Brockmeier et al., 2001).

The progression of PCV2 infection into PCV2- associated disease appears to be affected by both immune stimulation and co-infection with other respiratory pathogens (viral and bacterial) (Opriessnig et al., 2007). Vaccination with a commercial *M.hyopneumoniae* vaccine increased the severity of PCV-AD lymphoid lesions and the quantity of PCV2 genome in pig serum (Opriessnig et al., 2006).

Commensal flora of respiratory tract

The pig's response to pathogen challenge may be modulated by the microbial communities that colonise mucosal surfaces like the respiratory tract. The commensal bacteria are able to combat pathogens by competitive exclusion, through occupation of epithelial binding sites, and by competitive inhibition, through the production of antimicrobial substances (Bosch et al., 2013). Alternatively, or in combination, commensal bacteria may prevent or limit pathogen colonisation and/or proliferation by altering immune function (Bosch et al., 2013). Potential pathogens may be present in the microflora without causing disease if their growth is suitably controlled (Kich et al., 2000; Lowe et al., 2011). Respiratory pathogens were isolated from the BAL fluid of 56% of pigs from a high health herd without any clinical evidence or history of respiratory disease (Hennig-Pauka et al., 2007). A stable and balanced microflora has a greater ability to prevent disease, and disease may occur if an imbalance occurs in the microflora through exposure to bacterial pathogens, viral infection, antibiotic use, the host immune system or environmental factors (Bogaert et al., 2011).

While the majority of the microbial characterisation of the respiratory tract has been undertaken it humans, it is expected that the respiratory tract of pigs will have many similarities with humans. Recent human studies have demonstrated that the microbial population differs along the length of the respiratory tract (Bassis et al., 2015; Dickson et al., 2014). In healthy pig tonsils from two pig herds, the dominant bacterial families were *Pasteurellaceae, Porphromonadaceae, Bacteroidaceae* and *Prevotellaceae*, which made up 75% of the total microbial communities (Lowe et al., 2011). The dominant genera in both herds were *Actinobacillus, Haemophilus, Pasteurella, Porphyromonas, Fusobacterium, Bacteroides* and *Prevotella*.

The diversity of the microflora is also important in population stability and resistance to infection, with greater microbial diversity in the nasopharynges of healthy humans compared to rhinovirus infected humans (Allen et al., 2014). Decreased diversity in human nasal microflora was linked to colonisation by the respiratory pathogens *S. pneumoniae, H. influenzae* and *M. catarrhalis* (Pettigrew et al., 2012). Similarly, decreased microbial diversity in the oropharynx was correlated to the presence of the pathogen *Pseudomonas aeruginosa* (Klepac-Ceraj et al., 2010).

The commensal microbiota of the respiratory system can also be involved in competitive exclusion and/or inhibition to aid in the control of pathogenic bacteria and the prevention of disease. Commensal alpha-haemolytic streptococci inhibited the growth of pathogenic *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* in the nasopharynx of children (Tano et al., 2000). Increased abundance of the commensal *Lactococcus* spp. was negatively associated with nasal colonisation by the pathogens *S. pneumoniae, H. influenzae* and *M. catarrhalis* (Pettigrew et al., 2012). The human nostril microbiota also showed an inverse correlation between the abundance of commensal *Corynebacteriaceae* and *Propionibacteriaceae* families and the *Staphylococcaceae* family containing important pathogens (Lemon et al., 2010).

Differences in the microflora of the respiratory tract have been identified between individuals with and without respiratory disease. For example, viral infection was associated with decreased

Streptococcus spp. and increased *Haemophilus* spp. and *Moraxella* spp. in the oropharynx (Yi et al., 2014). Patients with asthma or chronic obstructive pulmonary disease (COPD) had a decreased abundance of commensal *Prevotella* spp. and increased abundance of pathogenic *Haemophilus* spp. in bronchial lavage (Hilty et al., 2010).

Commensal bacteria may also interact with the immune system to prevent or limit the severity of respiratory disease. Commensal bacteria may stimulate the immune system to specifically target pathogenic bacteria or may modulate the immune response to prevent further damage due to overstimulation by pathogens. Subclinical infection of the lungs with commensals, such as *Prevella* spp., may be able to prevent extensive lung damage by downregulating interleukin (IL)-12p70 production stimulated by *Haemophilus influenzae* in dendritic cells (Larsen et al., 2012).

Other factors which alter the respiratory microflora could lead to instability in the microbiome and predispose the respiratory system to pathogen colonisation, proliferation and clinical disease. Children treated with antibiotics had reduced microbial diversity in the oropharynx (Klepac-Ceraj et al., 2010), and antibiotics also alter the tonsillar microflora (Cafferkey et al., 1993). Season can also impact on the nasopharyngeal microbiota, with increased abundance of *Proteobacteria* and *Fusobacteria* in autumn/fall and decreased abundance of *Bacteroidetes* and *Firmicutes* (Bogaert et al., 2011).

Genetics and respiratory disease expression

Genetics almost certainly plays an important role in the expression of respiratory disease in pigs and the animal's ability to resist infection and disease. Relatively high heritabilities have been published for disease resistance to enzootic pneumonia (between 0.12 and 0.23) atrophic rhinitis (between 0.12 and 0.29) and pleurisy (h^2 = 0.13) (Lundeheim, 1979; Okamura et al., 2012). A genetic component to Glässers' susceptibility was proposed when statistical differences in the severity of Glässers disease (rectal temperatures and lesions) were observed in the offspring of 6 sires challenged with virulent *H.parasuis* (Blanco et al., 2008). However, none of these studies were able to demonstrate the mechanism for resistance.

The outcome of an infection depends upon resilience in the host as well as an ability to resist and tolerate infection. The earliest indicators of disease resistance in animals are innate immunity traits such as the activation of pathogen recognition receptors (PRR) on macrophages, neutrophils and epithelial cells lining the respiratory tract. Activation of these cells results in secretion of cytokines which induce inflammatory responses such as phagocytosis and compliment activation, all aimed at killing and clearing the pathogen from the host. A wide range of innate immunity traits were found to have moderate to high heritabilities in healthy pigs including cytokines (IL-1B, IL-6, IL-12, IFN-alpha), gamma-delta T cells, haptoglobin, phagocytes, natural killer cells, neutrophils, monocytes and macrophages (Flori et al., 2011).

As early as 24 hours post challenge with *H.parasuis*, differences in innate immunity gene expression were apparent between pigs with and without clinical signs of respiratory disease, including fever, lateral recumbency and laboured breathing. Genes differentially expressed included inflammatory and immune responses (toll-like receptors, complement and coagulation pathways), microtubule polymerization of macrophages, pathways for cell adhesion molecules and host cell receptor signalling (mitogen activated protein kinases, MAPK) (Wang et al., 2012b). Pro-inflammatory cytokine genes (especially IL-1β) were upregulated in both lungs and porcine alveolar macrophages from bronchoalveolar lavage of susceptible pigs (Wang et al., 2012b; Wilkinson et al., 2010). Susceptible pigs

also showed an imbalance in the signaling between pro and anti-inflammatory cytokines. In contrast, pigs resistant to *H.parasuis* showed increased expression of extracellular matrix proteins and TGF-beta signaling, suggesting that adhesion and entry to host cells was minimised and any tissue damage was repaired within 24 hours of infection (Wilkinson et al., 2010). By 72 hours post infection, increased expression of IgA in resistant pigs and reduced expression of antigen presentation genes (MHC I and II) in susceptible pigs indicated that both innate and acquired immune responses were involved in resistance to *H.parasuis*. It is interesting that the host responded to challenge by activating different immune and inflammatory pathways.

Potential resistance genes to infection with the Gram negative pathogens *A.pleuropneumoniae* and *H.parasuis* were reviewed, identifying a range of innate immunity traits that could affect expression of respiratory disease (Zhao et al., 2012). Genes for innate immunity pathways were upregulated in pigs with low APP disease and high production outcomes (Moser et al., 2008). More specifically, pigs infected with *A.pleuropneumoniae* showed upregulated expression of acute phase protein genes in the liver and immune activating and inflammation genes, cell signalling pathways and programmed cell death in inflamed lung tissue, including cytokines IL-1, IL-6, IL-8, LPS-binding protein, lactoferrin and pig MAP (Hedegaard et al., 2007; Skovgaard et al., 2010). Increased expression of metalloproteinase enzymes in alveolar epithelium suggests that *A.pleuropneumoniae* also affects the pig's immune response by degrading extracellular matrix proteins and modulating the activity of cytokines that traffic inflammatory cells to airways (Bruun et al., 2012). Variation in the innate immune response between pigs will certainly impact on the expression of respiratory disease following pathogen challenge.

Resistance to pathogens mediated by the host also includes adaptive immunity, where antibodies can neutralize or destroy bacterial pathogens by opsonisation and phagocytosis. Highly heritable adaptive immunity traits include antibody production (IgA and IgG), T helper cells and the anti-inflammatory cytokines (IL-2, IL-4, IL-10), and IFN-gamma (Flori et al., 2011). Heritabilities for increased humoral immunity have been reported for non-specific antibody production ($h^2 = 0.24$) (Okamura et al., 2012) and for *B.bronchiseptica*-specific antibodies following vaccination ($h^2 = 0.52 \pm 0.15$) (Meeker et al., 1987). Primary and secondary antibody responses to *Pasteurella multocida* show relatively high heritability estimates in turkeys (0.458 and 0.333 respectively) (Sacco et al., 1994). The hereditability of *A.pleuropneumoniae* specific antibodies was also demonstrated, where the offspring of sows with high APP antibody titres raised a higher antibody response to natural *A.pleuropneumoniae* exposure after maternal antibodies had waned (Sjölund et al., 2011). Selecting pigs with increased adaptive immunity may provide better protection against specific pathogens, but targeting innate immunity is more likely to improve the health status and disease resistance of pigs to more than one challenge.

6. Discussion

6.1 Investigate the impact of single or multiple respiratory pathogens on variation in slaughter weight, feed intake and variation in whole carcass composition

This study highlights the significant negative impact that multiple respiratory pathogens have on the expression of respiratory disease in growing pigs. *M. hyopneumoniae* infection prior to challenge with *P. multocida* significantly exacerbated the production, disease and body composition effects observed with a single *P. multocida* or *M. hyopneumoniae* challenge. The percentage area of lung affected with pneumonia was significantly greater in pigs challenged with both *M. hyopneumoniae* and *P. multocida*, with sub-acute lesions observed at 2.5 weeks after *P. multocida* challenge, and chronic lesions at 5.5 weeks. Although individual respiratory pathogens can affect production and carcase composition, pigs challenged with multiple pathogens were more severely affected with reduced mean pig weights, ADG, feed intake and gain to feed ratios. The severity of pneumonia was also associated with increased body temperatures over the first 2 weeks after secondary challenge with *P. multocida*. Pigs challenged with both pathogens were consistently the least active, and spent the least amount of time in eating behaviour. Pneumonia caused by combined infections also reduced the percentage of body fat by weight and increased % muscle.

Multiple pathogens also increased the variation within treatments in all of these production parameters. While reduced mean production measures lead to increased feed costs and delayed production; increased variation in production measures predominantly affects profitability by increasing the number of 'tail-ender' pigs, disrupting all-in-all-out production flows and increasing days to slaughter. These effects will be examined in more detail below.

6.1.1 Multiple respiratory pathogens increase feed costs and reduce profitability

The staggering 16kg reduction in the mean weight of pigs challenged with both *M. hyopneumoniae* and *P. multocida* relative to 20 week old control pigs illustrates the need for extra feed and time to allow these pigs to 'catch up' and reach market slaughter weights. It would take pigs challenged with multiple pathogens an additional 20 days to make up the lost 16kg (assuming ADG remains at 0.8 kg/day), reducing profits by $12.60 per pig (assuming feed costs $300/tonne and pigs continue to eat 2.1 kg feed per day). Alternately, if the producer chose to sell pigs below market weight to maintain production flows, a 16kg weight reduction in hot carcass weight would lead to reduced profits of $56 per pig (350c/kg x 16 kg). Over a batch of pigs this would equate to large losses, even if the pigs challenged with multiple pathogens had recovered completely after 17 weeks of age. While pigs challenged with either *M. hyopneumoniae* or *P. multocida* alone also suffered reduced weights (4 or 10kg respectively), the added feed costs to get these pigs to market weight would be substantially less, though all infections reduce potential profits.

Although feed intake was substantially reduced in the first week after challenge with Pasteurella (50% reduction compared to the week pre-challenge), feed intake recovered in pigs challenged with both pathogens by 4 weeks post challenge. However, these pigs were starting from a significantly lower body weight once feed intake improved, so required additional feed to reach market weight relative to uninfected pigs or pigs challenged with only one pathogen. The initial reduction in feed intake, followed by the later recovery is supported by the activity scores outlined in the final report of APL project #1870. Pigs challenged with both pathogens spent less time eating in the first 10 days post Pasteurella challenge, and more time in sedentary activity (sleeping, lying), corresponding to the most severe clinical period post infection.

Challenge with both *M. hyopneumoniae* and *P. multocida* led to rapid reductions in mean ADG within a week of the secondary *Pasteurella* challenge, illustrating the speed with which Pasteurella infection can negatively affect pig production. Slower growth of pigs leads to increased days to slaughter and therefore increasing feed costs, and reduced profitability. Surprisingly, rapid recovery in weight gain was also observed within three weeks of challenge; specifically under these clean environmental and hygiene conditions.

Under commercial conditions, pigs may be constantly challenged by pathogens, and are unlikely to face a single respiratory infection at one time point. In addition, they may be exposed to poor environmental conditions and infections from enteric pathogens, all of which will stimulate the pig's immune system and divert energy resources away from growth. Pigs in this trial had minimal exposure to the environmental risks most commonly associated with increased respiratory disease expression such as temperature fluctuations, dust, ammonia, bacterial endotoxins, poor ventilation and high stocking rates (Stärk 2000). It is therefore expected that pigs housed under commercial conditions, with multiple pathogen exposure and environmental stressors, would exceed the growth reductions seen in this trial (277g/d over four weeks), and would therefore suffer more losses in profitability.

Severe respiratory disease caused by combined infection with *M. hyopneumoniae* and *P. multocida* significantly reduced feed efficiency (gain to feed ratios), which means that infected pigs needed to eat more feed to reach the same target weights as uninfected pigs. The rapid decrease in gain to feed of pigs challenged with both pathogens was due to both significant reductions in ADG and feed intake. Surprisingly, pigs infected with multiple pathogens recovered feed efficiency relatively quickly.

Feed efficiency is typically reported as feed to gain where the smaller the value is (without being negative), the better the efficiency. However, in this trial we chose to express feed efficiency as gain to feed (the inverse), because the significant weight losses caused by pneumonia made feed to gain ratios negative. Gain to feed ratios allowed us to express the results using a unidirectional scale with increasing feed efficiency observed as increasing gain to feed ratios. A reduction in feed efficiency (gain:feed) by as little as 0.1 can have a significant effect of profitability, so controlling the expression of respiratory disease is critical. While single pathogens also reduced gain:feed ratios, the effect was not as severe as multiple pathogens.

The significant weight loss caused by multiple respiratory pathogens is due to both suppressed appetite (reduced feed intake) and redirection of ingested energy from muscle deposition to stimulation of the immune system and heat production (fever). The energy and protein demands of immunity include production and activation of leukocytes, cytokines, antibodies and bacterial peptides. In humans, severe infection causing sepsis reduced total body protein by 13% over 3 weeks and increased energy expenditure by 40% over 7 days (Plank and Hill 2000a; 2000b). *Actinobacillus pleuropneumoniae* infection in growing pigs increased energy requirements by 50% to 70% between day 2 and 5 post challenge, based on the severity of respiratory disease (Black *et al.*, 1998). Spurlock (1997) suggested that the reduced muscle protein synthesis associated with disease in rats may in part be caused by cytokine stimulation, which reduces growth hormone and insulin like growth factor levels and increases the release of stress hormones.

The increased severity of respiratory disease caused by multiple pathogens in this trial also significantly reduced the proportion of body fat and increased body muscle in the sub-acute period of pneumonia.

Although the reduction in body fat and specifically P2 fat may appear to be advantageous in producing a leaner carcass at slaughter, it also makes pigs more vulnerable to subsequent challenge with other pathogens and environmental stressors such as temperature, as energy reserves are reduced.

6.1.2 Multiple respiratory pathogens increase variation in production parameters, disrupting AIAO batch flows

Although reductions in mean body weight, ADG and feed intake caused by respiratory disease can increase days to slaughter, it is the variation in production parameters between affected pigs that really disrupts production flows and causes the associated profitability losses. In this trial, greater variation in ADG, feed intake, gain:feed and body composition (muscle and fat proportions) were observed in pigs challenged with multiple pathogens. There was also a trend towards greater variation in slaughter weights in pigs infected with multiple pathogens, although this was nullified by the unintended transmission of *Pasteurella* infection to some of the control pigs late in the trial. The proportion of lung tissue affected with pneumonia was also highly variable in pigs challenged with Pasteurella, particularly in the sub-acute period.

Variation in pig weights, growth, intake and feed efficiency are due to the fact that pigs vary in their response to pathogen challenge depending on factors such as their immune competence (genetics), the magnitude of immune stimulation, the presence of other pathogens and environmental stressors. In addition, the significant variation in volume of pneumonic lungs between pigs in the same treatment indicates that tissue damage varies in pigs given the same pathogenic challenge.

In this trial, the increased variation in weight, average daily gain and feed intake was observed at 2 to 3 weeks post Pasteurella challenge; a delay of one or two weeks after reduction in production was initially observed. This variation between pigs in the same treatment (both *M. hyopneumoniae* and *P. multocida* challenged) also indicates that the immune response to infection and disease varied between individual pigs. Although dynamic innate immune markers such as acute phase proteins or cytokines were not measured in this trial, it is possible that the variation was due to either increased early infection responses (pro-inflammatory) or increased early recovery responses (anti-inflammatory). The rapid recovery of some pigs in the third week after challenge with Pasteurella, also indicates that host immune factors play an important part in variation in disease expression and therefore profitability. Some pigs appeared to be able to change more rapidly from a pro-inflammatory cytokine response, where tissue damage is exacerbated, to an anti-inflammatory cytokine response, where immune stimulation is dampened down.

Variation in market weight impacts on profitability by increasing housing costs per pig, due to delayed emptying of sheds for light weight pigs in AIAO production. To avoid variation in market weight, producers often sort pigs on weight before sale. However, this frequent mixing of pigs can have significant impacts on both animal health and welfare. Producers can maintain AIAO production if they market different weight pigs to the most appropriate buyer. However, this is likely to cause additional sorting and transport costs.

The variation in feed intake, growth rates and weights caused by disease may be overcome with additional veterinary treatments or dietary formulations to help slow growing pigs recover prior to sale. However, all of these strategies ultimately lead to increased production costs and reduced profitability, so cost-benefit analyses need to be performed.

A large variation in carcase size, shape and composition will also affect profitability due to increased processing times for handling non-uniform carcases. In this trial, there was no increased variation in fat and muscle percentages or P2 muscle depth in pigs affected with pneumonia, though it is possible that this could change under field conditions. Additional environmental stressors, such as repeated challenge, temperature fluctuations, ammonia and dust particles, are likely to exacerbate the immune response to pathogen challenge and could lead to more significant variation in production parameters and body composition.

6.1.3 Conclusion

Respiratory diseases have significant negative impacts on pig slaughter weights, weight gains, feed intake, feed efficiency and body composition. All of these production losses lead to increased feed costs, extended days to slaughter and disruption of all-in-all-out production flows, ultimately causing reduced profitability of pig enterprises.

The expression of respiratory disease is impacted by many factors including challenge with multiple pathogens. In this trial, pigs challenged with multiple pathogens had more severe production losses and more variation in production measures than pigs challenged with only one pathogen or uninfected control pigs. The expression of respiratory disease is likely to be further exacerbated under commercial conditions where repeated pathogen challenge and environmental stressors are likely to increase variation in the pig's response to challenge, affecting production flows and meat processing. The AUSPIG modelling software could estimate the impact of increased variation under commercial conditions using the data from our experimental challenge trial.

6.2 Literature Review discussion: Factors that impact on the expression of respiratory disease and how to monitor them

Respiratory disease control relies on producers utilizing management tools to reduce factors that exacerbate disease like reducing stocking densities, improving air quality, ventilation, all-in-all-out production flows and minimal mixing of pigs, improved temperature control and hygiene and the need for disinfection between batches. Reducing the consequences of respiratory disease also relies on monitoring pigs for respiratory disease in real time to better evaluate disease management strategies.

It is unlikely that any single respiratory disease or respiratory pathogen assay will adequately measure respiratory disease, especially not in real time; thereby not providing producers with the opportunity to control disease. While coughing is a useful clinical sign of current disease, it is not yet indicative of the cause of disease (environmental or infectious) and may not correlate with disease severity. Further development of the electronic software is required to distinguish coughs from sick and healthy pigs, along with field evaluations. Although measuring lung lesions in real time by radiography, computed tomography or sonography provide accurate measures of disease severity, they are limited to research trials and are not practical measures on commercial farms. Estimation of lung gross pathology at slaughter is not pathognomonic for specific diseases, and is reported to under-estimate the extent of damage because lesions resolve before slaughter.

Measuring acute phase proteins or cytokines in serum do provide an accurate measure of the inflammatory damage associated with pathogen infection, but APPs and cytokines are also elevated in response to non-infectious injuries. They are also not specific for individual pathogens, so would need

to be supplemented with subsequent clinical signs (ie. dry non-productive cough), pathogen detection (PCR or culture) or serology. Cytokines and APPs are both very reactive, increasing rapidly after infection, so would require frequent monitoring. Very little data has been published on the detection of cytokines or APPs in oral fluids. If detection in oral fluids is sensitive enough, it would allow more frequent and cost-effective monitoring at the herd level. Correlations would also need to be demonstrated between cytokine and APP levels in oral fluids and other measures of respiratory disease.

Serology may be a useful tool to monitor seroconversion to specific bacterial pathogens on a herd basis, especially because seroconversion coincides with onset of coughing. However, serum antibodies to *M.hyopneumoniae* do not correlate with disease in individual pigs, and are an indicator of past infection. Antibody detection in serum or oral fluid samples is a cost-effective way to determine the prevalence of infection, and indicates that pigs are recovering from infection, but antibodies alone do not demonstrate immunity to re-infection. Oral fluids can monitor pig health in groups of pigs within a population, rather than sampling a relatively large number of individual pigs within a population and hence are a cost-effective tool. Oral fluids are collected on a cotton rope placed in the pen for a period of 20 to 30 minutes, by which time it is estimated that 70% of pigs within a pen of 25 pigs have had contact with the rope (Seddon et al., 2012). By sampling groups of animals, oral fluid testing has the potential to facilitate surveillance and detection of disease in a population. Oral fluids can contain both pathogens and the immune response to these pathogens (Prickett et al., 2008).

The major advantage of pathogen detection over serology is that it measures active infection, not past infection, so can measure pathogen loads in real time. Although pathogen numbers in BALF correlate well with disease, determining pathogen numbers in BALF is limited to experimental disease trials where BALF can be collected under anaesthesia of animals. Pathogen detection in oral fluids and nasal swabs appears to work well for viral pathogens and some secondary bacterial pathogens, but is less sensitive for the more important bacterial pathogens in pigs, namely *M.hyopneumoniae* and *A.pleuropneumoniae*. The detection of some pathogenic bacteria in nasal swabs and BALF is complicated by the fact that these pathogenic bacteria also occur in healthy pigs.

Quantifying the pathogen load of multiple pathogens in known volumes of air may appear to be a useful indicator of disease expression at the herd level, but field studies are still needed to correlate pathogen loads with other disease scores (coughing, serology or subsequent lung lesions at slaughter) and production.

A combination of disease and infection measures appears to be necessary to measure the severity of disease and to ensure the necessary sensitivity and specificity of diagnosis. As coughing is often the first clinical sign noted in pigs with respiratory disease, it needs to be measured in combination with more specific assays to diagnose and quantify the impact of disease. Coughing occurs at a similar time to the peak in pathogen load, so using qPCR assays to measure pathogen numbers in oral fluids or air samples may increase the specificity of diagnosis. Cytokine and acute phase protein responses are not pathogen specific and generally occur before coughing is noticed, so are unlikely to be useful in combined disease scores. Coughing scores and serology appear to be useful for supporting the diagnosis of enzootic pneumonia, but both measures have limitations and are either non-specific or insensitive.

As pathogen loads, antibiotic peptides, cytokines and antibodies in BALF appear to correlate well with disease severity, it might also be useful to find less technically difficult ways to capture fluids from the lower respiratory tracts.

Focussing on detecting potentiating pathogens may also be important for predicting potential respiratory disease outbreaks. The most common potentiating pathogens in Australian pigs are *M.hyopneumoniae*, influenza A virus and PCV2. Further studies linking these pathogens with later disease outbreaks may help in identifying disease predictors. As PCV2 and influenza A can be readily quantified in oral fluids, this may be the place to start monitoring to determine whether increased loads of prevalence of SIV, *M.hyopneumoniae* or PCV2 lead to increased susceptibility to *A.pleuropneumoniae, H.parasuis, P.multocida* and *S.suis*. Likewise monitoring of air samples for multiple pathogens may indicate threshold levels for more severe respiratory disease expression.

7. Implications & Recommendations

This study highlights the significant negative impact that multiple respiratory pathogens have on the expression of respiratory disease in growing pigs. Although infection with *M. hyopneumoniae* or *P. multocida* individually can affect production and carcase composition, pigs challenged with multiple pathogens were more severely affected with lesions and clinical signs of pneumonia, and also with reduced mean pig weights, ADG, feed intake and gain to feed ratios. The staggering 16kg reduction in the mean weight of pigs challenged with both *M. hyopneumoniae* and *P. multocida* relative to 20 week old healthy pigs illustrates the need for extra feed and time to allow these pigs to 'catch up' and reach market slaughter weights, reducing profits by as much as $12.60 per pig. While pigs challenged with either *M. hyopneumoniae* or *P. multocida* alone also suffered reduced weights, the added feed costs ($3.15 or $7.88 per pig respectively) had a smaller effect of reducing profits. The secondary challenge with *P.multocida* caused rapid reductions in ADG within a week, illustrating the need for continuous monitoring of herds to prevent production losses caused by disease.

The significant weight loss caused by multiple respiratory pathogens is due to both suppressed appetite (reduced feed intake) and redirection of ingested energy from muscle deposition to stimulation of the immune system and heat production (fever). The energy and protein demands for immunity have been estimated to be increased by 50% to 70% between day 2 and 5 post challenge with *Actinobacillus pleuropneumoniae*.

Pneumonia caused by combined infections also reduced the percentage of body fat by weight and increased % muscle. Although the reduction in body fat and specifically P2 fat may appear to be advantageous in producing a leaner carcass at slaughter, it also makes pigs more vulnerable to subsequent challenge with other pathogens and environmental stressors such as temperature, as energy reserves are reduced.

Pigs in this trial had minimal exposure to the environmental risks most commonly associated with increased respiratory disease expression such as temperature fluctuations, dust, ammonia, bacterial endotoxins, poor ventilation and high stocking rates, and therefore pigs housed under commercial conditions would be expected to suffer greater growth rate reductions than seen in this trial (277g/d over four weeks). The AUSPIG modelling software could estimate the impact of increased variation under commercial conditions using the data from our experimental challenge trial.

Importantly this study showed that multiple pathogens also increased the variation within treatments in all of the production parameters. While reduced ADG, FI and feed efficiency lead to increased feed costs and delayed production; increased variation in production predominantly affects profitability by increasing the number of 'tail-ender' pigs, disrupting all-in-all-out production flows and increasing days to slaughter. A large variation in carcase size, shape and composition will also affect profitability due to increased processing times for handling non-uniform carcases.

Variation in market weight impacts on profitability by increasing housing costs per pig, due to delayed emptying of sheds for light weight pigs in AIAO production. To avoid variation in market weight, producers often sort pigs on weight before sale. However, this frequent mixing of pigs can have significant impacts on both animal health and welfare. Producers can maintain AIAO production if they market different weight pigs to the most appropriate buyer. However, this is likely to cause additional sorting and transport costs.

The variation in feed intake, growth rates and weights caused by disease may be overcome with additional veterinary treatments or dietary formulations to help slow growing pigs recover prior to sale. However, all of these strategies ultimately lead to increased production costs and reduced profitability, so cost-benefit analyses need to be performed.

The expression of respiratory disease is exacerbated by multiple pathogens, but also by many other factors including stocking density, air quality, ventilation, all-in-all-out production flows and frequent mixing of pigs, temperature control and cleaning and disinfection between batches. The literature quantifies the increased risk of respiratory disease associated with many of these management factors, with biosecurity and air quality as two of the most important factors.

Reducing the consequences of respiratory disease relies on monitoring pigs for clinical signs and/or detection of pathogens in real time, to give producers the opportunity to control or prevent disease. Coughing is a useful clinical sign of current disease, but requires pathogen-specific detection to indicate the cause of disease. Measuring lung lesions at slaughter is not pathognomonic for specific diseases, and under-estimates the extent of damage because lesions resolve before slaughter. Serology is a useful tool to monitor pathogen-specific infections on a herd basis; however, serum antibodies are an indicator of past, not current infection. The detection of pathogen-specific IgA in oral fluids provides an earlier indicator of infection than serum IgG responses, and oral fluid samples are a cost-effective way to determine the timing of infection in pens of pigs. Oral fluid testing for either antibodies or pathogens has the potential to facilitate surveillance and detection of disease in a population. The sensitivity of pathogen detection of both *M.hyopneumoniae* and A.pleuropneumoniae in oral fluids is currently poor.

Quantifying the pathogen load of multiple pathogens in known volumes of air may be a useful indicator of disease expression at the herd level, but field studies are still needed to correlate pathogen loads with other disease scores (coughing, serology or subsequent lung lesions at slaughter) and production. This research may identify threshold levels for different pathogens that correlate with increased severity of respiratory disease. Focussing on quantifying potentiating pathogens, such as *M.hyopneumoniae*, influenza A virus and PCV2, may also be important for predicting potential respiratory disease outbreaks. Further studies linking these pathogens with subsequent disease outbreaks may help in identifying disease predictors. As PCV2 and influenza A can be readily quantified in oral fluids, this may be the place to start monitoring to determine whether increased loads of prevalence of SIV, *M.hyopneumoniae* or PCV2 lead to increased susceptibility to *A.pleuropneumoniae, H.parasuis, P.multocida* and *S.suis*. A combination of disease and infection measures appears to be necessary to measure the severity of disease and to ensure the necessary sensitivity and specificity of diagnosis. The literature suggests that coughing scores, along with the detection of IgA in oral fluids (or IgG in serum) and pathogen load in the environment may provide the best tools to diagnose and quantify the impact of disease.

8. Intellectual Property

No new intellectual property has been developed as part of this project, however, the new data on variation in production has yet to be published and could also be included into the AUSPIG model to quantify the costs associated with increased variation in production. Many of the results from the initial APL project #1870 investigating the production and carcass traits impacted by single or multiple respiratory pathogens have already been published (Eamens, 2006; Eamens et al., 2007). However, the effect of multiple respiratory pathogens on feed efficiency (expressed as gain to feed) have only recently been reported (Wyburn et al., 2015). The new analysis demonstrating the significant impact that respiratory disease has on variation has yet to be published, but a manuscript is in preparation. When this paper is approved for publication by both APL and the journal, the report should be freely available to the pig industry.

The second part of the report detailing factors that impact on respiratory disease is a review of the published literature, so no intellectual property exists in that report. It is likely that the risk factors and diagnostic assays identified in the literature review will be useful to vaccine companies and veterinarians in evaluating respiratory disease control.

9. Technical Summary

There were no new discoveries in methodology or equipment design, as this project was a desk-top study, which utilised data and analysis from previous pig respiratory disease research, both in Australia and overseas. However, the study did demonstrate the importance measuring both the mean and variation in production measures and more specifically using the Bartlett's test for significance of variation within and between treatments.

10. Publications Arising

Wyburn GL, Eamens GJ, Collins, D and Collins AM. 2015. Rapid changes occur in feed efficiency after infection with *Mycoplasma hyopneumoniae* and *Pasteurella multocida.* Animal Production Science **55**: 1568.

Publications in preparation:
Wyburn GL, Eamens GJ, and Collins AM. Increased variation in ADG, ADFI, G:F occurs in grower pigs affected with pneumonia. Journal of Animal Science.

11. Literature cited

Aalund, O., Willeberg, P., Mandrup, M., Riemann, H., 1976. Lung lesions at slaughter: associations to factors in the pig herd. Nordisk veterinaermedicin 28, 487-495.

Allen, E.K., Koeppel, A.F., Hendley, J.O., Turner, S.D., Winther, B., Sale, M.M., 2014. Characterization of the nasopharyngeal microbiota in health and during rhinovirus challenge. Microbiome 2, 22.

Amass, S., Baysinger, A. 2006. Swine disease transmission and prevention, In: Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.) Diseases of swine. Blackwell Publishing Ltd., Oxford, UK, 1075-1098.

Amass, S.F., Clark, L.K., van Alstine, W.G., Bowersock, T.L., Murphy, D.A., Knox, K.E., Albregts, S.R., 1994. Interaction of Mycoplasma hyopneumoniae and Pasteurella multocida infections in swine. Journal of the American Veterinary Medical Association 204, 102-107.

Amass, S.F., Clark, L.K., Wu, C.C., 1995. Source and timing of Streptococcus suis infection in neonatal pigs: Implications for early weaning procedures. Swine Health and Production 3, 189-193.

Artiushin, S., Minion, F.C., 1996. Arbitrarily primed PCR analysis of Mycoplasma hyopneumoniae field isolates demonstrates genetic heterogeneity. International journal of systematic bacteriology 46, 324-328.

Asai, T., Okada, M., Yokomizo, Y., Sato, S., Mori, Y., 1996. Suppressive effect of bronchoalveolar lavage fluid from pigs infected with Mycoplasma hyopneumoniae on chemiluminescence of porcine peripheral neutrophils. Veterinary Immunology and Immunopathology 51, 325-331.

Baarsch, M.J., Foss, D.L., Murtaugh, M.P., 2000. Pathophysiologic correlates of acute porcine pleuropneumonia. American journal of veterinary research 61, 684-690.

Bassis, C.M., Erb-Downward, J.R., Dickson, R.P., Freeman, C.M., Schmidt, T.M., Young, V.B., Beck, J.M., Curtis, J.L., Huffnagle, G.B., 2015. Analysis of the Upper Respiratory Tract Microbiotas as the Source of the Lung and Gastric Microbiotas in Healthy Individuals. Mbio 6.

Bello-Orti, B., Costa-Hurtado, M., Martinez-Moliner, V., Segalés, J., Aragon, V., 2014. Time course Haemophilus parasuis infection reveals pathological differences between virulent and non-virulent strains in the respiratory tract. Veterinary Microbiology 170, 430-437.

Beynon, L.M., Griffith, D.W., Richards, J.C., Perry, M.B., 1992. Characterization of the lipopolysaccharide O antigens of Actinobacillus pleuropneumoniae serotypes 9 and 11: antigenic relationships among serotypes 9, 11, and 1. Journal of Bacteriology 174, 5324-5331.

Black, J., Pluske, J. 2011. Review of innate immunite in pigs (Australian Pork Limited), 97.

Blanchard, B., Vena, M.M., Cavalier, A., Le Lannic, J., Gouranton, J., Kobisch, M., 1992. Electron microscopic observation of the respiratory tract of SPF piglets inoculated with Mycoplasma hyopneumoniae. Vet Microbiol 30, 329-341.

Blanco, I., Canals, A., Evans, G., Mellencamp, M.A., Cia, C., Deeb, N., Wang, L., Galina-Pantoja, L., 2008. Differences in susceptibility to Haemophilus parasuis infection in pigs. Canadian Journal of Veterinary Research 72, 228-235.

Bogaert, D., Keijser, B., Huse, S., Rossen, J., Veenhoven, R., van Gils, E., Bruin, J., Montijn, R., Bonten, M., Sanders, E., 2011. Variability and Diversity of Nasopharyngeal Microbiota in Children: A Metagenomic Analysis. PLoS ONE 6, e17035.

Bosch, A.A.T.M., Biesbroek, G., Trzcinski, K., Sanders, E.A.M., Bogaert, D., 2013. Viral and Bacterial Interactions in the Upper Respiratory Tract. PLoS Pathog 9, e1003057.

Bosse, J.T., Janson, H., Sheehan, B.J., Beddek, A.J., Rycroft, A.N., Kroll, J.S., Langford, P.R., 2002. Actinobacillus pleuropneumoniae: pathobiology and pathogenesis of infection. Microbes and infection / Institut Pasteur 4, 225-235.

Bossé, J.T., Li, Y., Angen, Ø., Weinert, L.A., Chaudhuri, R.R., Holden, M.T., Williamson, S.M., Maskell, D.J., Tucker, A.W., Wren, B.W., Rycroft, A.N., Langford, P.R., 2014. Multiplex PCR Assay for

Unequivocal Differentiation of Actinobacillus pleuropneumoniae Serovars 1 to 3, 5 to 8, 10, and 12. Journal of Clinical Microbiology 52, 2380-2385.

Bray, H.J., Giles, L.R., Walker, K.H., Gooden, J.M., Black, J.L. 1995. The effect of lung damage due to pleuropneumonia challenge on energy expenditure of growing pigs. In Manipulating Pig Production IV, 259.

Brockmeier, S.L., Halbur, P.G., Thacker, E.L. 2002. Porcine Respiratory Disease Complex, In: Brogden, K.A., Guthmiller, J.M. (Eds.) Polymicrobial Diseases. ASM Press, Washington (DC).

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., 2000. Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, Bordetella bronchiseptica, or a combination of both organisms in pigs. American journal of veterinary research 61, 892-899.

Brockmeier, S.L., Palmer, M.V., Bolin, S.R., Rimler, R.B., 2001. Effects of intranasal inoculation with Bordetella bronchiseptica, porcine reproductive and respiratory syndrome virus, or a combination of both organisms on subsequent infection with Pasteurella multocida in pigs. American journal of veterinary research 62, 521-525.

Bruun, C.S., Leifsson, P.S., Johansen, L.K., Jensen, H.E., Nielsen, J., Fredholm, M., 2012. Expression of matrix metalloproteinase-9 and -12 in porcine lung infections. J Comp Pathol 146, 253-257.

Burch, D.G.S. 2007. Cost of disease - enzootic pneumonia. In Pig World (United Kingdom, Lewis Business Media).

Cafferkey, M.T., Timon, C.I., O'Regan, M., Walsh, M., 1993. Effect of pre-operative antibiotic treatment on the bacterial content of the tonsil. Clin Otolaryngol Allied Sci 18, 512-516.

Calsamiglia, M., Collins, J.E., Pijoan, C., 2000. Correlation between the presence of enzootic pneumonia lesions and detection of Mycoplasma hyopneumoniae in bronchial swabs by PCR. Veterinary Microbiology 76, 299-303.

Cargill, C., Skirrow, S. 1997. Air quality in pig housing facilities. In: Proceedings of the Post-Graduate Foundation in Veterinary Science, Sydney, NSW, 85-103.

Caruso, J.P., Ross, R.F., 1990. Effects of Mycoplasma hyopneumoniae and Actinobacillus (Haemophilus) pleuropneumoniae infections on alveolar macrophage functions in swine. American journal of veterinary research 51, 227-231.

Carvalho, L.F., Segales, J., Pijoan, C., 1997. Effect of porcine reproductive and respiratory syndrome virus on subsequent Pasteurella multocida challenge in pigs. Vet Microbiol 55, 241-246.

Chiers, K., Donné, E., Van Overbeke, I., Ducatelle, R., Haesebrouck, F., 2002. Evaluation of serology, bacteriological isolation and polymerase chain reaction for the detection of pigs carrying Actinobacillus pleuropneumoniae in the upper respiratory tract after experimental infection. Veterinary Microbiology 88, 385-392.

Chiou, M.T., Jeng, C.R., Chueh, L.L., Cheng, C.H., Pang, V.F., 2000. Effects of porcine reproductive and respiratory syndrome virus (isolate tw91) on porcine alveolar macrophages in vitro. Vet Microbiol 71, 9-25.

Cho, W.-S., Chae, C., 2001. Genotypic Prevalence of apxIV in Actinobacillus Pleuropneumoniae Field Isolates. Journal of Veterinary Diagnostic Investigation 13, 175-177.

Cho, W.S., Jung, K., Kim, J., Ha, Y., Chae, C., 2005. Expression of mRNA encoding interleukin (IL)-10, IL-12p35 and IL-12p40 in lungs from pigs experimentally infected with Actinobacillus pleuropneumoniae. Vet Res Commun 29, 111-122.

Choi, C., Kwon, D., Jung, K., Ha, Y., Lee, Y.H., Kim, O., Park, H.K., Kim, S.H., Hwang, K.K., Chae, C., 2006. Expression of Inflammatory Cytokines in Pigs Experimentally Infected with Mycoplasma hyopneumoniae. Journal of Comparative Pathology 134, 40-46.

Choi, C., Kwon, D., Min, K., Chae, C., 1999. In-situ hybridization for the detection of inflammatory cytokines (IL-1, TNF-alpha and IL-6) in pigs naturally infected with Actinobacillus pleuropneumoniae. J Comp Pathol 121, 349-356.

Christensen, G., 1981. [Pleuropneumonia in swine due to Haemophilus pleuropneumonia s. parahaemolyticus. II. Studies on the epidemiology and the relation to chronic pleuritis (pleural scars) in baconers (author's transl)]. Nordisk veterinaermedicin 33, 236-249.

Christensen, N.H., 1995. Evaluation of the effects of enzootic pneumonia in pigs on weight gain and days to slaughter under New Zealand conditions. New Zealand veterinary journal 43, 146-148.

Ciprian, A., Cruz, T.A., de la Garza, M., 1994. Mycoplasma hyopneumoniae: interaction with other agents in pigs, and evaluation of immunogens. Archives of medical research 25, 235-239.

Ciprian, A., Pijoan, C., Cruz, T., Camacho, J., Tortora, J., Colmenares, G., Lopez-Revilla, R., de la Garza, M., 1988. Mycoplasma hyopneumoniae increases the susceptibility of pigs to experimental Pasteurella multocida pneumonia. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 52, 434-438.

Collins, A.M., Wyburn, G.L. 2015. Factors that impact on the expression of respiratory disease in pigs (NSW Department of Primary Industries).

Costa, G., Oliveira, S., Torrison, J., 2012. Detection of Actinobacillus pleuropneumoniae in oral-fluid samples obtained from experimentally infected pigs. Journal of Swine Health and Production 20, 78-81.

Davies, P.R., Bahnson, P.B., Grass, J.J., Marsh, W.E., Dial, G.D., 1995. Comparison of methods for measurement of enzootic pneumonia lesions in pigs. American journal of veterinary research 56, 09-14.

DeBey, M.C., Ross, R.F., 1994. Ciliostasis and loss of cilia induced by Mycoplasma hyopneumoniae in porcine tracheal organ cultures. Infect Immun 62, 5312-5318.

Deblanc, C., Robert, F., Pinard, T., Gorin, S., Queguiner, S., Gautier-Bouchardon, A., Ferre, S., Garraud, J., Cariolet, R., Brack, M., Simon, G., 2013. Pre-infection of pigs with Mycoplasma hyopneumoniae induces oxidative stress that influences outcomes of a subsequent infection with a swine influenza virus of H1N1 subtype. Veterinary Microbiology 162, 643-651.

Dickson, R.P., Erb-Downward, J.R., Huffnagle, G.B., 2014. Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. Lancet Respir Med 2, 238-246.

Djordjevic, S.P., Eamens, G.J., Romalis, L.F., Nicholls, P.J., Taylor, V., Chin, J., 1997. Serum and mucosal antibody responses and protection in pigs vaccinated against Mycoplasma hyopneumoniae with vaccines containing a denatured membrane antigen pool and adjuvant. Australian veterinary journal 75, 504-511.

Donham, K.J., 1991. Association of environmental air contaminants with disease and productivity in swine. American journal of veterinary research 52, 1723-1730.

Dreyfus, A., Schaller, A., Nivollet, S., Segers, R.P., Kobisch, M., Mieli, L., Soerensen, V., Hussy, D., Miserez, R., Zimmermann, W., Inderbitzin, F., Frey, J., 2004. Use of recombinant ApxIV in serodiagnosis of Actinobacillus pleuropneumoniae infections, development and prevalidation of the ApxIV ELISA. Vet Microbiol 99, 227-238.

Dritz, S.S., Owen, K.Q., Goodband, R.D., Nelssen, J.L., Tokach, M.D., Chengappa, M.M., Blecha, F., 1996. Influence of lipopolysaccharide-induced immune challenge and diet complexity on growth performance and acute-phase protein production in segregated early-weaned pigs. J Anim Sci 74, 1620-1628.

Drummond, J.G., Curtis, S.E., Meyer, R.C., Simon, J., Norton, H.W., 1981. Effects of atmospheric ammonia on young pigs experimentally infected with Bordetella bronchiseptica. American journal of veterinary research 42, 963-968.

Drummond, J.G., Curtis, S.E., Simon, J., 1978. Effects of atmospheric ammonia on pulmonary bacterial clearance in the young pig. American journal of veterinary research 39, 211-212.

Drummond, J.G., Curtis, S.E., Simon, J., Norton, H.W., 1980. Effects of Aerial Ammonia on Growth and Health of Young Pigs. Journal of Animal Science 50, 1085-1091.

Dubreuil, J.D., Jacques, M., Mittal, K.R., Gottschalk, M., 2000. Actinobacillus pleuropneumoniae surface polysaccharides: their role in diagnosis and immunogenicity. Animal health research reviews / Conference of Research Workers in Animal Diseases 1, 73-93.

Eamens, G.J. 2006. Quantifying respiratory disease impact for Auspig (New South Wales Department of Primary Industries).

Eamens, G.J., Gonsalves, J.R., Whittington, A.M., Turner, B., 2008. Serological responses to two serovar-independent ELISA antigens of Actinobacillus pleuropneumoniae in Australian commercial pig herds. Australian veterinary journal 86, 465-472.

Eamens, G.J., Gonsalves, J.R., Whittington, A.M., Turner, B., 2012. Evaluation of serovar-independent ELISA antigens of Actinobacillus pleuropneumoniae in pigs following vaccination or experimental challenge with respiratory pathogens and natural A. pleuropneumoniae serovar 1 challenge. Australian veterinary journal 90, 490-498.

Eamens, G.J., James, K.J., Barchia, I.M., Giles, L.R. 2007. Use of computer tomography in live pigs to measure the impact of pneumonia on carcass composition. In Manipulating Pig Production XI, Paterson, J.E., Baker, J.A., eds. (Brisbane, Australia, Australasian Pig Science Association), 60.

Eamens, G.J., Jenkins, C., Fell, S., Gonsalves, J.R., Woolley, L. 2011. Effects of mycoplasmal pneumonia during the first weeeks after challenge. In Manipulating Pig Production XIII (Australasian Pig Science Association), 214.

Eckersall, P.D., Saini, P.K., McComb, C., 1996. The acute phase response of acid soluble glycoprotein, alpha(1)-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. Vet Immunol Immunopathol 51, 377-385.

Eisenmenger, M. 2006. Warm, dry and draft free. In: Proceedings Allen D Leman swine conference, 41-44.

Erlandson, K.R., Evans, R.B., Thacker, B.J., Wenger, M.W., Thacker, E.L., 2005. Evaluation of three serum antibody enzyme-linked immunosorbent assays for Mycoplasma hyopneumoniae. Journal of Swine Health and Production 13, 198-203.

Escobar, J., Van Alstine, W.G., Baker, D.H., Johnson, R.W., 2002. Growth performance and whole-body composition of pigs experimentally infected with Mycoplasma hyopneumoniae. Journal of animal science 80, 384-391.

Eurell, T.E., Bane, D.P., Hall, W.F., Schaeffer, D.J., 1992. Serum haptoglobin concentration as an indicator of weight gain in pigs. Canadian Journal of Veterinary Research 56, 6-9.

Fablet, C., Marois, C., Kobisch, M., Madec, F., Rose, N., 2010. Estimation of the sensitivity of four sampling methods for Mycoplasma hyopneumoniae detection in live pigs using a Bayesian approach. Vet Microbiol 143, 238-245.

Fano, E., Pijoan, C., Dee, S., Deen, J., 2012. Longitudinal assessment of two Mycoplasma hyopneumoniae enzyme-linked immunosorbent assays in challenged and contact-exposed pigs. Journal of Veterinary Diagnostic Investigation 24, 383-387.

Feld, N.C., Qvist, P., Ahrens, P., Friis, N.F., Meyling, A., 1992. A monoclonal blocking ELISA detecting serum antibodies to Mycoplasma hyopneumoniae. Vet Microbiol 30, 35-46.

Fittipaldi, N., Broes, A., Harel, J., Kobisch, M., Gottschalk, M., 2003. Evaluation and Field Validation of PCR Tests for Detection of Actinobacillus pleuropneumoniae in Subclinically Infected Pigs. Journal of Clinical Microbiology 41, 5085-5093.

Flesja, K.I., Forus, I.B., Solberg, I., 1982. Pathological lesions in swine at slaughter. V. Pathological lesions in relation to some environmental factors in the herds. Acta Vet Scand 23, 169-183.

Flori, L., Gao, Y., Laloë, D., Lemonnier, G., Leplat, J.-J., Teillaud, A., Cossalter, A.-M., Laffitte, J., Pinton, P., de Vaureix, C., Bouffaud, M., Mercat, M.-J., Lefèvre, F., Oswald, I.P., Bidanel, J.-P., Rogel-Gaillard, C., 2011. Immunity Traits in Pigs: Substantial Genetic Variation and Limited Covariation. PLoS ONE 6, e22717.

Frey, J., 1995. Virulence in Actinobacillus pleuropneumoniae and RTX toxins. Trends in Microbiology 3, 257-261.

Frey, J. 2003. Detection, Identification, and Subtyping of Actinobacillus pleuropneumoniae, In: Sachse, K., Frey, J. (Eds.) PCR detection of microbial pathogens. Methods in molecular biology, 87-95.

Giesert, A.-L., Balke, W.-T., Jahns, G., 2011. Probabilistic analysis of coughs in pigs to diagnose respiratory infections. Landbauforschung - VTI Agriculture and forestry research 61, 237-242.

Gomes Neto, J.C., Strait, E.L., Raymond, M., Ramirez, A., Minion, F.C., 2014. Antibody responses of swine following infection with Mycoplasma hyopneumoniae, M. hyorhinis, M. hyosynoviae and M. flocculare. Vet Microbiol 174, 163-171.

Goodell, C.K., Prickett, J., Kittawornrat, A., Zhou, F., Rauh, R., Nelson, W., O'Connell, C., Burrell, A., Wang, C., Yoon, K.J., Zimmerman, J.J., 2013. Probability of detecting influenza A virus subtypes H1N1 and H3N2 in individual pig nasal swabs and pen-based oral fluid specimens over time. Vet Microbiol 166, 450-460.

Goodwin, R.F.W., Hodgson, R.G., Whittlestone, P., Woodhams, R.L., 1969. Some experiments relating to artificial immunity in enzootic pneumonia of pigs. Epidemiology & Infection 67, 465-476.

Gottschalk, M., Altman, E., Lacouture, S., De Lasalle, F., Dubreuil, J.D., 1997. Serodiagnosis of swine pleuropneumonia due to Actinobacillus pleuropneumoniae serotypes 7 and 4 using long-chain lipopolysaccharides. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 61, 62-65.

Gram, T., Ahrens, P., Andreasen, M., Nielsen, J.P., 2000. An Actinobacillus pleuropneumoniae PCR typing system based on the apx and omlA genes — evaluation of isolates from lungs and tonsils of pigs. Veterinary Microbiology 75, 43-57.

grosse Beilage, E., Rohde, N., Krieter, J., 2009. Seroprevalence and risk factors associated with seropositivity in sows from 67 herds in north-west Germany infected with Mycoplasma hyopneumoniae. Preventive Veterinary Medicine 88, 255-263.

Habrun, B., Bilić, V., Humski, A., 1998. Comparison of ELISA and 2-META assays used in serological diagnosis of infection with Actinobacillus pleuropneumoniae serotypes 2 and 4–7 in breeding pigs in Croatia. Preventive Veterinary Medicine 36, 179-186.

Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R., Decostere, A., 2004. Efficacy of vaccines against bacterial diseases in swine: what can we expect? Vet Microbiol 100, 255-268.

Hall, W., Molitor, T.W., Joo, H.S., Pijoan, C., 1989. Comparison of protective immunity and inflammatory responses of pigs following immunization with different Actinobacillus pleuropneumoniae preparations with and without adjuvants. Vet Immunol Immunopathol 22, 175-186.

Hamilton, T.D., Roe, J.M., Hayes, C.M., Jones, P., Pearson, G.R., Webster, A.J., 1999. Contributory and exacerbating roles of gaseous ammonia and organic dust in the etiology of atrophic rhinitis. Clinical and diagnostic laboratory immunology 6, 199-203.

Hamilton, T.D., Roe, J.M., Webster, A.J., 1996. Synergistic role of gaseous ammonia in etiology of Pasteurella multocida-induced atrophic rhinitis in swine. J Clin Microbiol 34, 2185-2190.

Hedegaard, J., Skovgaard, K., Mortensen, S., Sørensen, P., Jensen, T.K., Hornshøj, H., Bendixen, C., Heegaard, P.M.H., 2007. Molecular characterisation of the early response in pigs to experimental infection with Actinobacillus pleuropneumoniae using cDNA microarrays. Acta Vet Scand 49, 11-11.

Heegaard, P.M., Klausen, J., Nielsen, J.P., Gonzalez-Ramon, N., Pineiro, M., Lampreave, F., Alava, M.A., 1998. The porcine acute phase response to infection with Actinobacillus pleuropneumoniae. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology 119, 365-373.

Hennessy, K.J., Iandolo, J.J., Fenwick, B.W., 1993. Serotype identification of Actinobacillus pleuropneumoniae by arbitrarily primed polymerase chain reaction. J Clin Microbiol 31, 1155-1159.

Hennig-Pauka, I., Bremerich, S., Nienhoff, H., Schröder, C., Verspohl, J., Strutzberg-Minder, K., Ganter, M., Waldmann, K.H., Blecha, F., Beyerbach, M., Gerlach, G.F., 2007. Respiratory Disease Markers in Porcine Bronchoalveolar Lavage Fluid. Journal of Veterinary Medicine Series A 54, 434-440.

Hennig-Pauka, I., Koch, R., Hoeltig, D., Gerlach, G.-F., Waldmann, K.-H., Blecha, F., Brauer, C., Gasse, H., 2012. PR-39, a porcine host defence peptide, is prominent in mucosa and lymphatic tissue of the respiratory tract in healthy pigs and pigs infected with actinobacillus pleuropneumoniae. BMC Research Notes 5, 539-539.

Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., Davies, J., Ervine, A., Poulter, L., Pachter, L., Moffatt, M.F., Cookson, W.O., 2010. Disordered microbial communities in asthmatic airways. PLoS One 5, e8578.

Hoeltig, D., Hennig-Pauka, I., Thies, K., Rehm, T., Beyerbach, M., Strutzberg-Minder, K., Gerlach, G.F., Waldmann, K.H., 2009. A novel Respiratory Health Score (RHS) supports a role of acute lung damage and pig breed in the course of an Actinobacillus pleuropneumoniae infection. BMC veterinary research 5, 14.

Hurnik, D., Dohoo, I.R., Bate, L.A., 1994. Types of farm management as risk factors for swine respiratory disease. Preventive Veterinary Medicine 20, 147-157.

Hurnik, D., Hanna, P.E., Dohoo, I.R., 1993. Evaluation of rapid gross visual appraisal of swine lungs at slaughter as a diagnostic screen for enzootic pneumonia. Canadian Journal of Veterinary Research 57, 37-41.

Inzana, T.J., 1991. Virulence properties of Actinobacillus pleuropneumoniae. Microbial pathogenesis 11, 305-316.

Inzana, T.J., Fenwick, B., 2001. Serologic Detection of Actinobacillus pleuropneumoniae in Swine by Capsular Polysaccharide-Biotin-Streptavidin Enzyme-Linked Immunosorbent Assay. Journal of Clinical Microbiology 39, 1279-1282.

Inzana, T.J., Ma, J., Workman, T., Gogolewski, R.P., Anderson, P., 1988. Virulence properties and protective efficacy of the capsular polymer of Haemophilus (Actinobacillus) pleuropneumoniae serotype 5. Infect Immun 56, 1880-1889.

Jacobson, M., Lindberg, J.E., Lindberg, R., Segerstad, C.H., Wallgren, P., Fellstrom, C., Hulten, C., Jensen-Waern, M., 2001. Intestinal cannulation: model for study of the midgut of the pig. Comparative medicine 51, 163-170.

Keller, H. 1976. Subclincial M.suispneumoniae infections in SPF herds. In: Proceedings of the 4th IPVS Congress, Ames, Iowa, 11.

Kich, J.D., Piffer, I.A., Barcellos, D., Guidoni, A.L., Klein, C.S., Favero, M.B.B., Vizotto, R., 2000. Methods to isolate NAD-dependent bacteria from the upper respiratory tract of health pigs. Arquivo Brasileiro De Medicina Veterinaria E Zootecnia 52, 1-6.

Klepac-Ceraj, V., Lemon, K.P., Martin, T.R., Allgaier, M., Kembel, S.W., Knapp, A.A., Lory, S., Brodie, E.L., Lynch, S.V., Bohannan, B.J., Green, J.L., Maurer, B.A., Kolter, R., 2010. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and Pseudomonas aeruginosa. Environmental microbiology 12, 1293-1303.

Kobisch, M., Blanchard, B., Le Potier, M.F., 1993. Mycoplasma hyopneumoniae infection in pigs: duration of the disease and resistance to reinfection. Vet Res 24, 67-77.

Kurth, K.T., Hsu, T., Snook, E.R., Thacker, E.L., Thacker, B.J., Minion, F.C., 2002. Use of a Mycoplasma hyopneumoniae nested polymerase chain reaction test to determine the optimal sampling sites in swine. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 14, 463-469.

Labarque, G., Van Gucht, S., Nauwynck, H., Van Reeth, K., Pensaert, M., 2003. Apoptosis in the lungs of pigs infected with porcine reproductive and respiratory syndrome virus and associations with the production of apoptogenic cytokines. Vet Res 34, 249-260.

Larsen, J.M., Steen-Jensen, D.B., Laursen, J.M., Sondergaard, J.N., Musavian, H.S., Butt, T.M., Brix, S., 2012. Divergent pro-inflammatory profile of human dendritic cells in response to commensal and pathogenic bacteria associated with the airway microbiota. PLoS One 7, e31976.

Lee, C., Giles, L.R., Bryden, W.L., Downing, J.L., Owens, P.C., Kirby, A.C., Wynn, P.C., 2005. Performance and endocrine responses of group housed weaner pigs exposed to the air quality of a commercial environment. Livestock Production Science 93, 255-262.

Lemon, K.P., Klepac-Ceraj, V., Schiffer, H.K., Brodie, E.L., Lynch, S.V., Kolter, R., 2010. Comparative Analyses of the Bacterial Microbiota of the Human Nostril and Oropharynx. mBio 1.

Liu, J., Chen, X., Tan, C., Guo, Y., Chen, Y., Fu, S., Bei, W., Chen, H., 2009. In vivo induced RTX toxin ApxIVA is essential for the full virulence of Actinobacillus pleuropneumoniae. Vet Microbiol 137, 282-289.

Loftager, M.K., Eriksen, L., Nielsen, R., 1993. Antibodies against Actinobacillus pleuropneumoniae serotype 2 in mucosal secretions and sera of infected pigs as demonstrated by an enzyme-linked immunosorbent assay. Research in Veterinary Science 54, 57-62.

Lorenzo, H., Quesada, O., Assuncao, P., Castro, A., Rodriguez, F., 2006. Cytokine expression in porcine lungs experimentally infected with Mycoplasma hyopneumoniae. Vet Immunol Immunopathol 109, 199-207.

Lowe, B.A., Marsh, T.L., Isaacs-Cosgrove, N., Kirkwood, R.N., Kiupel, M., Mulks, M.H., 2011. Microbial communities in the tonsils of healthy pigs. Veterinary Microbiology 147, 346-357.

Lundeheim, N., 1979. Genetic analysis of respiratory diseases in pigs. Acta Agriculturae Scandinavica 29, 209-215.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B., Verbeke, W., Viaene, J., de Kruif, A., 1999. Effect of vaccination against Mycoplasma hyopneumoniae in pig herds with an all-in/all-out production system. Vaccine 17, 1024-1034.

Maes, D., Segales, J., Meyns, T., Sibila, M., Pieters, M., Haesebrouck, F., 2008. Control of Mycoplasma hyopneumoniae infections in pigs. Veterinary Microbiology 126, 297-309.

Maes, D., Verdonck, M., Deluyker, H., de Kruif, A., 1996. Enzootic pneumonia in pigs. Veterinary Quarterly 18, 104-109.

Magowan, E., McCann, M.E.E., Beattie, V.E., McCracken, K.J., Henry, W., Smyth, S., Bradford, R., Gordon, F.J., Mayne, C.S., 2007. Investigation of growth rate variation between commercial pig herds. animal 1, 1219-1226.

Marenda, M., Markham, P., Watt, A. 2014. Real-time detection of airborne pathogens in the piggery (Asia Pacific Centre for Animal Health, Univeristy of Melbourne), 40.

Marois, C., Dory, D., Fablet, C., Madec, F., Kobisch, M., 2010. Development of a quantitative Real-Time TaqMan PCR assay for determination of the minimal dose of Mycoplasma hyopneumoniae strain 116 required to induce pneumonia in SPF pigs. Journal of Applied Microbiology 108, 1523-1533.

Marois, C., Gottschalk, M., Morvan, H., Fablet, C., Madec, F., Kobisch, M., 2009. Experimental infection of SPF pigs with Actinobacillus pleuropneumoniae serotype 9 alone or in association with Mycoplasma hyopneumoniae. Vet Microbiol 135, 283-291.

Marois, C., Le Carrou, J., Kobisch, M., Gautier-Bouchardon, A.V., 2007. Isolation of Mycoplasma hyopneumoniae from different sampling sites in experimentally infected and contact SPF piglets. Veterinary Microbiology 120, 96-104.

Meeker, D.L., Rothschild, M.F., Christian, L.L., Warner, C.M., Hill, H.T., 1987. Genetic Control of Immune Response to Pseudorabies and Atrophic Rhinitis Vaccines: II: Comparison of Additive Direct and Maternal Genetic Effects1,2. J. Anim. Sci. 64, 414-419.

Minion, F.C., Adams, C., Hsu, T., 2000. R1 region of P97 mediates adherence of Mycoplasma hyopneumoniae to swine cilia. Infect Immun 68, 3056-3060.

Møller, K., Nielsen, R., Andersen, L.V., Kilian, M., 1992. Clonal analysis of the Actinobacillus pleuropneumoniae population in a geographically restricted area by multilocus enzyme electrophoresis. Journal of Clinical Microbiology 30, 623-627.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., 1995a. Enzootic pneumonia: comparison of cough and lung lesions as predictors of weight gain in swine. Canadian Journal of Veterinary Research 59, 197-204.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., Parker, K.M., 1995b. Seroepidemiologic study of natural transmission of Mycoplasma hyopneumoniae in a swine herd. Preventive Veterinary Medicine 21, 323-337.

Moser, R.J., Reverter, A., Lehnert, S.A., 2008. Gene expression profiling of porcine peripheral blood leukocytes after infection with Actinobacillus pleuropneumoniae. Vet Immunol Immunopathol 121, 260-274.

Muller, R.D., Abbott, P.B., 1986. Estimating the cost of respiratory disease in hogs. Animal Nutrition and Health 41, 30-34.

Nathues, H., Spergser, J., Rosengarten, R., Kreienbrock, L., Grosse Beilage, E., 2012. Value of the clinical examination in diagnosing enzootic pneumonia in fattening pigs. Veterinary journal (London, England : 1997) 193, 443-447.

Nielsen, R., 1993. Pathogenicity and immunity studies of Haemophilus parasuis serotypes. Acta Vet Scand 34, 193-198.

Noyes, E.P., Feeney, D.A., Pijoan, C., 1990a. Comparison of the effect of pneumonia detected during lifetime with pneumonia detected at slaughter on growth in swine. Journal of the American Veterinary Medical Association 197, 1025-1029.

Noyes, E.P., Jacobson, L., Mendez, A., Pijoan, C. 1990b. Study of immunological parameters of pigs housed with cold air drats or fluctuating temperatures. In: Proceedings of the 11th IPVS Congress, Lausanne, 25.

Okamura, T., Onodera, W., Tayama, T., Kadowaki, H., Kojima-Shibata, C., Suzuki, E., Uemoto, Y., Mikawa, S., Hayashi, T., Awata, T., Fujishima-Kanaya, N., Mikawa, A., Uenishi, H., Suzuki, K., 2012. A genome-wide scan for quantitative trait loci affecting respiratory disease and immune capacity in Landrace pigs. Animal Genetics 43, 721-729.

Olvera, A., Ballester, M., Nofrarías, M., Sibila, M., Aragon, V., 2009. Differences in phagocytosis susceptibility in Haemophilus parasuis strains. Veterinary Research 40, 24.

Olvera, A., Cerda-Cuellar, M., Aragon, V., 2006. Study of the population structure of Haemophilus parasuis by multilocus sequence typing. Microbiology (Reading, England) 152, 3683-3690.

Opriessnig, T., Giménez-Lirola, L.G., Halbur, P.G., 2011. Polymicrobial respiratory disease in pigs. Animal Health Research Reviews 12, 133-148.

Opriessnig, T., Halbur, P.G., Yu, S., Thacker, E.L., Fenaux, M., Meng, X.J., 2006. Effects of the timing of the administration of Mycoplasma hyopneumoniae bacterin on the development of lesions associated with porcine circovirus type 2. The Veterinary record 158, 149-154.

Opriessnig, T., Hemann, M., Johnson, J.K., Heinen, S., Gimenez-Lirola, L.G., O'Neill, K.C., Hoang, H., Yoon, K.J., Gottschalk, M., Halbur, P.G., 2013. Evaluation of diagnostic assays for the serological detection of Actinobacillus pleuropneumoniae on samples of known or unknown exposure. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 25, 61-71.

Opriessnig, T., Meng, X.-J., Halbur, P.G., 2007. Porcine Circovirus Type 2–Associated Disease: Update on Current Terminology, Clinical Manifestations, Pathogenesis, Diagnosis, and Intervention Strategies. Journal of Veterinary Diagnostic Investigation 19, 591-615.

Opriessnig, T., Thacker, E.L., Yu, S., Fenaux, M., Meng, X.J., Halbur, P.G., 2004. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and porcine circovirus type 2. Veterinary pathology 41, 624-640.

Otagiri, Y., Asai, T., Okada, M., Uto, T., Yazawa, S., Hirai, H., Shibata, I., Sato, S., 2005. Detection of Mycoplasma hyopneumoniae in lung and nasal swab samples from pigs by nested PCR and culture methods. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 67, 801-805.

Panyasing, Y., Goodell, C.K., Wang, C., Kittawornrat, A., Prickett, J.R., Schwartz, K.J., Ballagi, A., Lizano, S., Zimmerman, J.J., 2014. Detection of influenza A virus nucleoprotein antibodies in oral fluid specimens from pigs infected under experimental conditions using a blocking ELISA. Transboundary and emerging diseases 61, 177-184.

Paradis, S.E., Dubreuil, D., Rioux, S., Gottschalk, M., Jacques, M., 1994. High-molecular-mass lipopolysaccharides are involved in Actinobacillus pleuropneumoniae adherence to porcine respiratory tract cells. Infect Immun 62, 3311-3319.

Patience, J.F., Engele, K., Beaulieu, A.D., Gonyou, H.W., Zijlstra, R.T., 2004. Variation: Costs and consequences. Advances in Pork Production 15, 257-266.

Payne, H.G., Mullan, B.P., Trezona, M., Frey, B. 1999. A review - variation in pig production and preformance. In Manipulating pig production VII, Cranwell, P.D., ed. (Adelaide), 13-26.

Pettigrew, M.M., Laufer, A.S., Gent, J.F., Kong, Y., Fennie, K.P., Metlay, J.P., 2012. Upper respiratory tract microbial communities, acute otitis media pathogens, and antibiotic use in healthy and sick children. Applied and environmental microbiology 78, 6262-6270.

Pointon, A.M. 1990. REspiratory diseases, In: Gardner, J., Dunkin, A.C., Lloyd, L.C. (Eds.) Pig Production in Australia. Butterworth, 191-199.

Pointon, A.M., Heap, P., McCloud, P., 1985. Enzootic pneumonia of pigs in South Australia--factors relating to incidence of disease. Australian veterinary journal 62, 98-101.

Pointon, A.M., Morrison, R.B., Hill, G., Dargatz, D., Dial, G. 1990. Monitoring pathology in slaughtered stock: Guidelines for selecting sample size and interpreting results. In: Proceedings of the 11th IPVS Congress, Lausanne, 393.

Pointon, A.M., Sloane, M., 1984. An abattoir survey of the prevalence of lesions of enzootic pneumonia of pigs in South Australia. Australian veterinary journal 61, 408-409.

Prickett, J., Kim, W., Simer, R., Yoon, K.J., Zimmerman, J., 2008. Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. Journal of Swine Health and Production 16, 86-91.

Prickett, J.R., Johnson, J., Murtaugh, M.P., Puvanendiran, S., Wang, C., Zimmerman, J.J., Opriessnig, T., 2011. Prolonged Detection of PCV2 and Anti-PCV2 Antibody in Oral Fluids Following Experimental Inoculation. Transboundary and emerging diseases 58, 121-127.

Rautiainen, E., Nyberg, M., Levonen, K. 1996. The use of sow colostrum samples for regular herd health control of Mycoplasma hyopneumoniae infection. In: Proceedings of the 14th IPVS Congress, Bologna, 219.

Ro, L.H., Ross, R.F., 1983. Comparison of Mycoplasma hyopneumoniae strains by serologic methods. American journal of veterinary research 44, 2087-2094.

Robertson, J.F., Wilson, D., Smith, W.J., 1990. Atrophic rhinitis: the influence of the aerial environment. Animal Science 50, 173-182.

Rosendal, S., Mitchell, W.R., 1983. Epidemiology of Haemophilus pleuropneumoniae infection in pigs: A survey of Ontario Park Producers, 1981. Canadian journal of comparative medicine 47, 1-5.

Sacco, R.E., Nestor, K.E., Saif, Y.M., Tsai, H.J., Anthony, N.B., Patterson, R.A., 1994. Genetic analysis of antibody responses of turkeys to Newcastle disease virus and Pasteurella multocida vaccines. Poultry Science 73, 1169-1174.

Schaller, A., Djordjevic, S.P., Eamens, G.J., Forbes, W.A., Kuhn, R., Kuhnert, P., Gottschalk, M., Nicolet, J., Frey, J., 2001. Identification and detection of Actinobacillus pleuropneumoniae by PCR based on the gene apxIVA. Vet Microbiol 79, 47-62.

Schaller, A., Kuhn, R., Kuhnert, P., Nicolet, J., Anderson, T.J., MacInnes, J.I., Segers, R.P., Frey, J., 1999. Characterization of apxIVA, a new RTX determinant of Actinobacillus pleuropneumoniae. Microbiology (Reading, England) 145 (Pt 8), 2105-2116.

Scheepens, C.J.M., 1996. Climatic stress in swine: hazards for health. The Pig Journal 37, 130-136.

Scheidt, A.B., Cline, T.R., Clark, L.K., Mayrose, V.B., Van Alstine, W.G., Diekman, M.A., Singleton, W.L., 1995. The effect of all-in-all-out srowing- finishing on the health of pigs. Swine Health and Production 3, 202-205.

Seddon, Y.M., Guy, J.H., Edwards, S.A., 2012. Optimising oral fluid collection from groups of pigs: effect of housing system and provision of ropes. Veterinary journal (London, England : 1997) 193, 180-184.

Seymour, L.M., Jenkins, C., Deutscher, A.T., Raymond, B.B., Padula, M.P., Tacchi, J.L., Bogema, D.R., Eamens, G.J., Woolley, L.K., Dixon, N.E., Walker, M.J., Djordjevic, S.P., 2012. Mhp182 (P102) binds fibronectin and contributes to the recruitment of plasmin(ogen) to the Mycoplasma hyopneumoniae cell surface. Cellular microbiology 14, 81-94.

Shibata, I., Okada, M., Urono, K., Samegai, Y., Ono, M., Sakano, T., Sato, S., 1998. Experimental dual infection of cesarean-derived, colostrum-deprived pigs with Mycoplasma hyopneumoniae and pseudorabies virus. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 60, 295-300.

Sibila, M., Bernal, R., Torrent, D., March, R., Llopart, D., Riera, P., Calsamiglia, M. 2006. Effect of Mycoplasma hyopneumoniae sow vaccination on colonization, seroconversion and presence of enzootic pneumoniacompatible lung lesions. In: Proceedings of the 19th IPVS Congress, Copenhagen, Denmark, 103.

Sibila, M., Calsamiglia, M., Vidal, D., Badiella, L., Aldaz, Á., Jensen, J.C., 2004. Dynamics of Mycoplasma hyopneumoniae infection in 12 farms with different production systems. Canadian Journal of Veterinary Research 68, 12-18.

Sibila, M., Nofrarías, M., López-Soria, S., Segalés, J., Valero, O., Espinal, A., Calsamiglia, M., 2007. Chronological study of Mycoplasma hyopneumoniae infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. Veterinary Microbiology 122, 97-107.

Sitjar, M., Noyes, E.P., Simon, X., Pijoan, C., 1996. Relationships among seroconversion to Mycoplasma hyopneumoniae, lung lesions, and production parameters in pigs. Swine Health and Production 4, 273-277.

Sjölund, M., Zoric, M., Persson, M., Karlsson, G., Wallgren, P., 2011. Disease patterns and immune responses in the offspring to sows with high or low antibody levels to Actinobacillus pleuropneumoniae serotype 2. Research in Veterinary Science 91, 25-31.

Skovgaard, K., Mortensen, S., Boye, M., Hedegaard, J., Heegaard, P.M., 2010. Hepatic gene expression changes in pigs experimentally infected with the lung pathogen Actinobacillus pleuropneumoniae as analysed with an innate immunity focused microarray. Innate immunity 16, 343-353.

Smart, N.L., Miniats, O.P., MacInnes, J.I., 1988. Analysis of Haemophilus parasuis isolates from southern Ontario swine by restriction endonuclease fingerprinting. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 52, 319-324.

Sorensen, V., Ahrens, P., Barfod, K., Feenstra, A.A., Feld, N.C., Friis, N.F., Bille-Hansen, V., Jensen, N.E., Pedersen, M.W., 1997. Mycoplasma hyopneumoniae infection in pigs: duration of the disease and evaluation of four diagnostic assays. Vet Microbiol 54, 23-34.

Sorensen, V., Barfod, K., Feld, N.C., Vraa-Andersen, L., 1993. Application of enzyme-linked immunosorbent assay for the surveillance of Mycoplasma hyopneumoniae infection in pigs. Revue scientifique et technique de l'Office International des Epizooties 12, 593-604.

Stark, K.D., Keller, H., Eggenberger, E., 1992. Risk factors for the reinfection of specific pathogen-free pig breeding herds with enzootic pneumonia. The Veterinary record 131, 532-535.

Stärk, K.D.C., 2000. Epidemiological Investigation of the Influence of Environmental Risk Factors on Respiratory Diseases in Swine—A Literature Review. The Veterinary Journal 159, 37-56.

Stärk, K.D.C., Keller, H., Eggenberger, E. 1992. Climatic conditions and airborne transmission of Mycoplasma hyopneumoniae. In: Proceedings of the 12th IPVS Congress, The Hague, 559.

Stärk, K.D.C., Nicolet, J., Frey, J., 1998. Detection of Mycoplasma hyopneumoniae by Air Sampling with a Nested PCR Assay. Applied and environmental microbiology 64, 543-548.

Strait, E.L., Madsen, M.L., Minion, F.C., Christopher-Hennings, J., Dammen, M., Jones, K.R., Thacker, E.L., 2008. Real-Time PCR Assays To Address Genetic Diversity among Strains of Mycoplasma hyopneumoniae. Journal of Clinical Microbiology 46, 2491-2498.

Straw, B.E., Shin, S.J., Yeager, A.E., 1990. Effect of pneumonia on growth rate and feed efficiency of minimal disease pigs exposed to Actinobacillus pleuropneumoniae and Mycoplasma hyopneumoniae. Preventive Veterinary Medicine 9, 287-294.

Straw, B.E., Tuovinen, V.K., Bigras-Poulin, M., 1989. Estimation of the cost of pneumonia in swine herds. Journal of the American Veterinary Medical Association 195, 1702-1706.

Syrovets, T., Lunov, O., Simmet, T., 2012. Plasmin as a proinflammatory cell activator. Journal of leukocyte biology 92, 509-519.

Tano, K., Grahn-Hakansson, E., Holm, S.E., Hellstrom, S., 2000. Inhibition of OM pathogens by alpha-hemolytic streptococci from healthy children, children with SOM and children with rAOM. International journal of pediatric otorhinolaryngology 56, 185-190.

Tascon, R.I., Vazquez-Boland, J.A., Gutierrez-Martin, C.B., Rodriguez-Barbosa, I., Rodriguez-Ferri, E.F., 1994. The RTX haemolysins ApxI and ApxII are major virulence factors of the swine pathogen Actinobacillus pleuropneumoniae: evidence from mutational analysis. Molecular microbiology 14, 207-216.

Tegetmeyer, H.E., Jones, S.C., Langford, P.R., Baltes, N., 2008. ISApl1, a novel insertion element of Actinobacillus pleuropneumoniae, prevents ApxIV-based serological detection of serotype 7 strain AP76. Vet Microbiol 128, 342-353.

Thacker, B. 2006. Mycoplasmal diseases, In: Straw, B.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.) Diseases of Swine. Blackwell Publishing Ltd, Oxford, UK, 701-717.

Thacker, E.L., Halbur, P.G., Ross, R.F., Thanawongnuwech, R., Thacker, B.J., 1999. Mycoplasma hyopneumoniae Potentiation of Porcine Reproductive and Respiratory Syndrome Virus-Induced Pneumonia. Journal of Clinical Microbiology 37, 620-627.

Thacker, E.L., Thacker, B.J., Janke, B.H., 2001. Interaction between Mycoplasma hyopneumoniae and Swine Influenza Virus. Journal of Clinical Microbiology 39, 2525-2530.

Thacker, E.L., Thacker, B.J., Kuhn, M., Hawkins, P.A., Waters, W.R., 2000. Evaluation of local and systemic immune responses induced by intramuscular injection of a Mycoplasma hyopneumoniae bacterin to pigs. American journal of veterinary research 61, 1384-1389.

Thanawongnuwech, R., Thacker, B., Halbur, P., Thacker, E.L., 2004. Increased production of proinflammatory cytokines following infection with porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae. Clinical and diagnostic laboratory immunology 11, 901-908.

Thanawongnuwech, R., Thacker, E.L., Halbur, P.G., 1997. Effect of porcine reproductive and respiratory syndrome virus (PRRSV) (isolate ATCC VR-2385) infection on bactericidal activity of porcine pulmonary intravascular macrophages (PIMs): in vitro comparisons with pulmonary alveolar macrophages (PAMs). Vet Immunol Immunopathol 59, 323-335.

Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential production of proinflammatory cytokines: in vitro PRRSV and Mycoplasma hyopneumoniae co-infection model. Vet Immunol Immunopathol 79, 115-127.

Tobias, T.J., Bouma, A., Daemen, A.J., Wagenaar, J.A., Stegeman, A., Klinkenberg, D., 2013. Association between transmission rate and disease severity for Actinobacillus pleuropneumoniae infection in pigs. Vet Res 44, 2.

Tobias, T.J., Bouma, A., Klinkenberg, D., Daemen, A.J., Stegeman, J.A., Wagenaar, J.A., Duim, B., 2012. Detection of Actinobacillus pleuropneumoniae in pigs by real-time quantitative PCR for the apxIVA gene. Veterinary journal (London, England : 1997) 193, 557-560.

Tokach, M. 2004. Dealing with variation in market weight. In Advances in Pork Production (Prairie Swine Center, Saskatoon), 281-290.

Van Alstine, W.G. 2012. Respiratory system, In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.) Deiseases of Swine. John Wiley and Sons, Chichester, West Sussex, UK, 348-362.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A., Haesebrouck, F., 2003. Evaluation of virulence of Mycoplasma hyopneumoniae field isolates. Vet Microbiol 97, 177-190.

Wallgren, P., Beskow, P., Fellstrom, C., Renstrom, L.H., 1994. Porcine lung lesions at slaughter and their correlation to the incidence of infections by Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae during the rearing period. Zentralblatt fur Veterinarmedizin. Reihe B. Journal of veterinary medicine. Series B 41, 441-452.

Wang, S.J., Liu, W.J., Yang, L.G., Sargent, C.A., Liu, H.B., Wang, C., Liu, X.D., Zhao, S.H., Affara, N.A., Liang, A.X., Zhang, S.J., 2012a. Effects of FUT1 gene mutation on resistance to infectious disease. Mol Biol Rep 39, 2805-2810.

Wang, Y., Liu, C., Fang, Y., Liu, X., Li, W., Liu, S., Liu, Y., Liu, Y., Charreyre, C., Audonnet, J.-C., Chen, P., He, Q., 2012b. Transcription analysis on response of porcine alveolar macrophages to Haemophilus parasuis. BMC Genomics 13, 68-68.

Wilkie, B.N., Mallard, B.A. 1998. Multi-trait selection for immune response; A possible alternative strategy for enhanced livestock health and productivity, In: Wiseman, J. (Ed.) Progress in Pig Science. Nottingham University Press, Nottingham, 29-38.

Wilkinson, J., Sargent, C., Galina-Pantoja, L., Tucker, A., 2010. Gene expression profiling in the lungs of pigs with different susceptibilities to Glasser's disease. BMC Genomics 11, 455.

Woolley, L.K., Fell, S., Gonsalves, J.R., Walker, M.J., Djordjevic, S.P., Jenkins, C., Eamens, G.J., 2012. Evaluation of clinical, histological and immunological changes and qPCR detection of Mycoplasma hyopneumoniae in tissues during the early stages of mycoplasmal pneumonia in pigs after experimental challenge with two field isolates. Veterinary Microbiology 161, 186-195.

Woolley, L.K., Fell, S.A., Djordjevic, S.P., Eamens, G.J., Jenkins, C., 2013. Plasmin activity in the porcine airways is enhanced during experimental infection with Mycoplasma hyopneumoniae, is positively correlated with proinflammatory cytokine levels and is ameliorated by vaccination. Veterinary Microbiology 164, 60-66.

Wyburn, G.L., Eamens, G.J., Collins, D., Collins, A.M., 2015. Rapid changes occur in feed efficiency after infection with Mycoplasma hyopneumoniae and Pasteurella multocida. Animal Production Science 55, 1568.

Yi, H., Yong, D., Lee, K., Cho, Y.J., Chun, J., 2014. Profiling bacterial community in upper respiratory tracts. BMC Infect Dis 14, 583.

Young, T.F., Ross, R.F., 1987. Assessment of antibody response of swine infected with Mycoplasma hyopneumoniae by immunoblotting. American journal of veterinary research 48, 651-656.

Zhang, H., Lunney, J.K., Baker, R.B., Opriessnig, T., 2011. Cytokine and chemokine mRNA expression profiles in tracheobronchial lymph nodes from pigs singularly infected or coinfected with porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (MHYO). Veterinary Immunology and Immunopathology 140, 152-158.

Zhao, S., Zhu, M., Chen, H., 2012. Immunogenomics for identification of disease resistance genes in pigs: a review focusing on Gram-negative bacilli. Journal of Animal Science and Biotechnology 3, 34-34.

Zimmerman, D.R., Spear, M.L., Switzer, W.P., 1973. Effect of Mycoplasma hyopneumoniae infections, pyrantel treatment and protein nutrition on performance of pigs exposed to soil containing Ascaris suum ova. J Anim Sci 36, 894-897.