Breeding Focus 2014 – Improving Resilience

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Published by

Animal Genetics and Breeding Unit

University of New England

Animal Genetics and Breeding Unit

University of New England

Armidale NSW 2351

Australia

http://agbu.une.edu.au

ISBN: 978-1-921-597-65-7

eISBN: 978-1-921-597-66-4

Cover design by Susan Joyal

Book design by Kathy Dobos

First published, 2014

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On-farm measures to monitor the health and immune status of pigs

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Abstract

Resilience is defined as the ability of an animal to recover from disease and the associated production and profitability losses. Laboratories have developed tools to quantify pathogen load and populations of protective bacteria, and to measure the immune response in immunized or diseased pigs. The expression of disease is affected by pathogen numbers, the presence of potentiating or multiple pathogens and virulence factors associated with the pathogen. However disease expression is also affected by host factors including genetics and immune responses and environmental factors such as air quality, temperature and humidity. All of these factors can be measured and, if correlated with production parameters, may prove useful to monitor disease expression and resilience in pig herds.

Introduction

An animal's response to infection can be characterised as tolerant, resistant or resilient. Resistance has been defined by breeders as the ability of an animal to exert control over the pathogen or parasite lifecycle, which can be measured by pathogen load (Bishop, 2012). Resilience is differentiated from resistance by measuring the productivity or performance outcome following an infection challenge. Tolerance is the net impact on performance of a given level of infection (Bishop, 2012). Tolerance, resilience and resistance to disease are obviously closely related, but in this paper I have focussed on resilience, defined as the ability of an animal to recover from disease and the associated production and profitability losses. Laboratories have developed tools to quantify pathogen load and populations of protective bacteria, and to measure the immune response in immunized or diseased pigs. The expression of disease is affected by pathogen numbers, the presence of potentiating or multiple pathogens and virulence factors associated with the pathogen. However disease expression is also affected by host factors including genetics and immune responses and environmental factors such as air quality, temperature and humidity. All of these factors can be measured and, if correlated with production parameters, may prove useful to monitor disease expression and resilience in pig herds. This paper will examine how these health and disease monitoring tools can be used to measure disease severity and recovery from disease.

Measuring disease and recovery

On farm, producers are largely limited to measuring disease severity and recovery, rather than resilience. However, resilience is linked to disease recovery, which is also linked to disease severity. Often producers use antibiotics, probiotics, prebiotics or vaccines to reduce disease severity or to aid in recovery, but these treatments may also enhance the host's ability to resist re-infections or concurrent infections. Although disease, recovery and resilience are closely associated, the measurement of these states is different. On farm, disease is most frequently monitored by observing pigs for clinical signs, which vary significantly depending on where the infection occurs. Scouring, inappetence and anorexia are the predominant clinical signs of intestinal diseases, whereas respiratory diseases appear clinically as coughing, sneezing, nasal discharge, fever, lethargy and arthritis. Pigs affected with diseases of the nervous system typically present with fever, lack of coordination and paralysis. In systemic diseases, such as Erysipelas, clinical signs include arthritis, skin lesions and endocarditis. Some pathogens like porcine circovirus (PCV2) are associated with several manifestations of disease including enteritis, dermatitis, reproductive disorders and kidney disease. Reproductive diseases are most likely to be observed as abortions, mummified foetuses, stillbirths, reduced farrowing rates, and delayed returns to oestrous (Zimmerman *et al.*, 2012).

Poor growth and reduced feed intake are observed in many diseases where clinical signs are present. Energy is diverted from muscle deposition and growth to immune responses during disease and recovery periods in pigs. Stimulation of the immune system impacts on the pig's metabolism, sequestering nutrients and energy 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. Even in sub-clinical infections, energy is being directed away from growth, towards tissue repair and immune activation.

Disease severity can be measured in both live and dead pigs with a combination of clinical signs, pathology and pathogen culture and a range of ante mortem diagnostic assays. Histopathology allows the extent of tissue damage to be visualised, along with the pathogens causing these pathological changes (Zimmerman *et al*., 2012). Pathogens can be isolated from specific organs (liver, kidney, lungs, intestine, brain) or from body fluids. In mucosal infections of both the respiratory and intestinal tract, local immune responses induced by the pathogen can also be measured, including antibody secretion, activated innate and adaptive immune cells and their products such as cytokines. Likewise, immunoglobulin detection in foetal fluids of aborted foetuses indicates whether reproductive failure may be due to infections such as parvovirus and Bungowannah virus (Leslie-Steen and Kirkbride, 1983; Mengeling, 1983; Finlaison *et al.*, 2009).

However, more information about disease severity and recovery can be collected from live animals over time than necropsied animals at a single time point. Pathogens infecting live animals can be isolated from faeces, blood, saliva, nasal discharge, skin lesions, arthritic joints and cerebral spinal fluids (Sims, 1996). Disease severity and recovery can be quantified by pathogen

detection using ante mortem techniques such as culture, antigen capture immunoassays or the polymerase chain reaction (PCR). The immune response to infection can also be used to quantify disease severity and recovery. Circulating antibodies to pathogens are generally measured in blood but can also be detected in faeces, saliva (oral fluids) and cerebral spinal fluid. Inflammatory responses, which are commonly part of the disease process, can be measured in blood and oral fluids, either as acute phase proteins or cytokines.

Disease expression is often separated into acute, clinical and sub-clinical manifestations, which vary in the onset, duration and severity of clinical signs, immune responses and pathogen levels. Clinical disease is characterised by increased pathogen numbers often before or at about the same time as clinical signs are apparent. Immune responses are activated at the site of infection including complement fixation, activation of antigen presenting cells, recruitment of phagocytes, production of pro-inflammatory cytokines and acute phase proteins. These are followed later by adaptive immune responses including production of IgM, IgA and IgG at the site of infection and in the circulatory system. In sub-clinical infections, clinical signs of disease won't be apparent, but pathogen numbers can be quantified and the immune system is still activated.

Some of the same assays that measure disease severity can also be used to monitor disease recovery. Pathogen load in the diseased animal will reach a peak, often coinciding with clinical disease, and will then decline as the host's immune response fights infection. Innate immune responses are usually the first line of attack against infection, including phagocytosis, the production of pro-inflammatory cytokines and acute phase proteins. However the recovery period is characterised by a combination of anti-inflammatory cytokines that down regulate the production of pro-inflammatory cytokines, protecting the host from over-stimulation of the immune system, and antibody production to the specific pathogen. ELISAs are used to measure concentrations of specific antibodies in body fluids, and the less specific acute phase proteins and cytokines.

The temporal pattern of infection, disease and recovery can be best illustrated with examples such as the enteric pig pathogens *Lawsonia intracellularis* and *Brachyspira hyodysenteriae*. The first sign of *Lawsonia intracellularis* infection in pigs is faecal excretion of these bacteria within 7 days post challenge (Collins and Love, 2007). Diarrhoea is evident about one week later and reduced weight gains only appear another week after that. It is at this point, 21 days post infection, that pathological changes in the intestine are at their peak, although minimal changes may be visible as early as 14 days post infection (Guedes and Gebhart, 2003; Collins and Love, 2007). It is also at this time that circulating antibodies (IgG) to *Lawsonia intracellularis* and cytokines are detected, although an earlier *Lawsonia intracellularis*- specific IgG and IgM response and pro-inflammatory cytokines can be found in the intestinal mucosa between 9 and 17 days post infection (Nogueira *et al*., 2013). Intestinal lesions typically begin to recover by 28 days post infection, but the circulating immune response is detectable for another six weeks (Guedes and Gebhart, 2003; Collins and Love, 2007). Immunity to *Lawsonia intracellularis* requires about 21 days following either vaccination or natural challenge (Kroll *et al.*, 2004; Collins and Love, 2007). The pro-inflammatory cytokine IFN-γ plays a role in limiting

bacterial infection and inducing immunity in many diseases. In normal mice challenged with *Lawsonia intracellularis*, infection and intestinal lesions resolved within 21 days, but in mice without the IFN- γ receptor, lesions failed to resolve after 35 days (Smith *et al.*, 2000).

Serum from pigs experimentally affected with swine dysentery demonstrate elevated levels of pro-inflammatory cytokines (IL-1ß, TNF-α and IL-6) and acute phase proteins (serum amyloid A) at early and peak dysentery periods; whereas the anti-inflammatory cytokine IL-10 only appear during the recovery period (Kruse *et al.*, 2008). Monitoring disease severity and recovery therefore has a role for both pro and anti-inflammatory cytokines. It is critical that pro-inflammatory cytokines are induced to help fight infection, but anti-inflammatory cytokines aid in recovery and could be a useful measure for disease recovery and resilience.

Monitoring both disease and recovery from disease will depend on the nature of disease expression. In animals that die rapidly with acute disease, increases in pathogen number can be monitored but pigs may be dead before significant immune responses are measureable, ie Erysipelas, *Actinobacillus pleuropneumoniae*. Similar diagnostic tools and clinical samples can be used to measure disease recovery. As pigs recover from disease, tissue lesions resolve and the normal tissue architecture returns, although some evidence of previous disease may be evident. Disease recovery can be measured by reduced pathogen numbers, which coincide with an elevated antibody response and the production of anti-inflammatory cytokines, a feedback mechanism to suppress the previously activated pro-inflammatory cytokines. Resilient animals will raise an immune response to infection, but won't over-accentuate the inflammatory immune response, and will have good feedback mechanisms to suppress immune stimulation when the pathogen attack has been dealt with.

Measuring pathogen load

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. Average daily gain, gross pathology and histopathology of the enteric disease ileitis all correlate well with pathogen load of the causative agent *Lawsonia intracellularis* (Pedersen *et al.*, 2012a; Pedersen *et al.*, 2012b; Collins and Barchia, 2014). Likewise the severity of atrophic rhinitis correlates with the numbers of the respiratory pathogen *Pasteurella multocida* isolated from tonsils and nasal membranes (Hamilton *et al.*, 1996; Hamilton *et al.*, 1999). 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. Loads of more than 10^7 PCV2 genome copies per millilitre of serum differentiate PCV2 infection from PCV2 associated disease (Brunborg *et al.*, 2004; Olvera *et al.*, 2004). In pigs sub-clinically affected with ileitis (no scouring), *Lawsonia intracellularis* infection can impact on feed intake, weight gain and feed conversion ratio. The ability to quantify the critical threshold, or num-

ber of *Lawsonia intracellularis* that cause production losses in individual pigs and commercial herds enables producers to evaluate disease control strategies to avoid production losses (Fig. 1). Large reductions in average daily gain (131 g/day) occurred over the three week disease period when grower pigs shed more than 10^7 *Lawsonia intracellularis* per gram of faeces at the peak in infection (day 14), with smaller reductions (15 g/day) when pigs shed one log less *Lawsonia intracellularis* (Collins and Barchia, 2014).

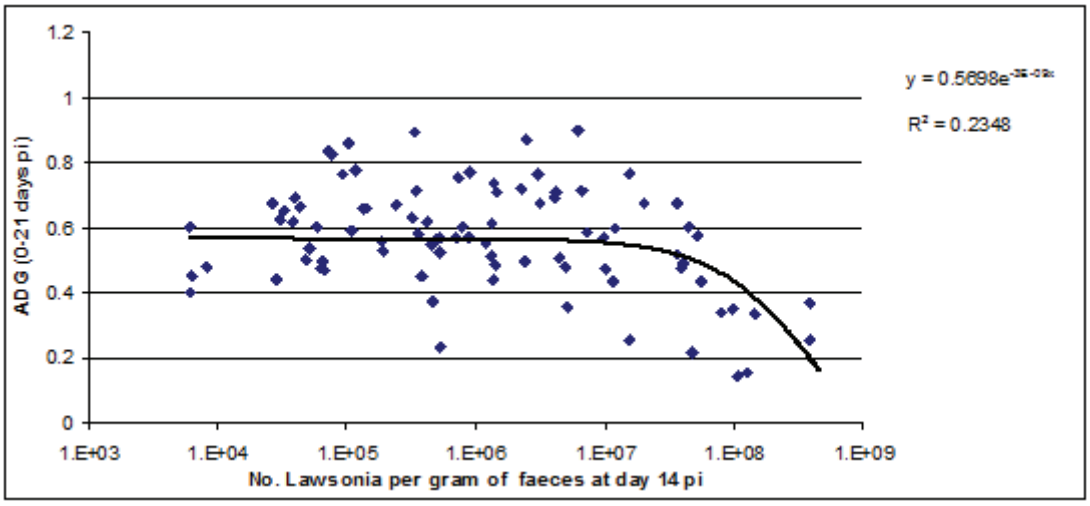

Figure 1. The critical threshold of Lawsonia intracellularis that causes average daily gain (ADG) reductions in grower pigs can be estimated from the exponential regression of ADG over 21 days post infection (pi) versus Lawsonia intracellularis numbers in faeces at 14 days pi in 101 individual pigs.

Measuring the impact of environment on disease

In commercial pig herds, both environmental (humidity, dust, ammonia, carbon dioxide, endotoxin) and host factors (genetics, immune response and the microbiome) can also impact on disease severity. Elevated concentrations of ammonia and hydrogen sulphide gases and particulate materials like dust, skin and airborne bacteria negatively affect the health and growth of pigs (Robertson *et al.*, 1990; Cargill and Skirrow, 1997). 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). Indeed, 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 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 *Escherichia coli* (Drummond *et al.*, 1978), an important factor in recovery and resilience from disease. Increased phagocytosis and superoxide anion were observed at 10 weeks in pigs removed at 2 weeks of age from a *Mycoplasma hyopneumoniae* and PRRS (porcine reproductive and respiratory syndrome) positive farm, although these pigs showed no evidence of either infection over 20 weeks (Jolie *et al.*, 1999). 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. However, environmental monitoring may be less useful in measuring recovery from disease or resilience.

Measuring the immune response to disease

In some disease presentations, the immune response to infection may provide an equally useful measure of tissue damage and disease severity as pathogen load. This is particularly true in respiratory infection where more than one pathogen may cause disease. Concurrent infections of *Mycoplasma hyopneumoniae* with *Pasteurella multocida, Bordetella bronchiseptica, Haemophilus parasuis* or *Actinobacillus pleuropneumoniae* can all cause enzootic pneumonia in pigs. While *Mycoplasma hyopneumoniae* infection alone has a minimal impact on pig growth and body composition, pigs infected with *Pasteurella multocida* after a *Mycoplasma hyopneumoniae* challenge suffered significantly more severe reductions in weight gain, feed intake and liveweight at 20 weeks of age (Eamens *et al.*, 2007). The combined infection also significantly increased pneumonia severity (lung lesion and clinical scores), lethargy, and altered body composition in the grower and finisher phases. *Mycoplasma hyopneumoniae* infection also potentiated infection by porcine circovirus type 2 (PCV2), PRRS and swine influenza virus, causing fever, more severe pneumonic lesions and increased expression of pro-inflammatory cytokines in the pig's lungs (Thacker *et al.*, 1999; Zhang *et al.*, 2011; Deblanc *et al.*, 2013; Woolley *et al.*, 2013). 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).

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), immunisation (Dritz *et al.*, 1996) and infection (Heegaard *et al.*, 1998). They are considered a good measure of infection because levels elevate rapidly and conversely they have a short half-life in serum and they are detected in sub-clinically affected animals (Eurell *et al.*, 1992). However, they are not a specific measure of disease, and high variation between in-

dividuals makes it difficult to interpret what elevated concentrations mean. Like other immune responses, the production of APPs are a cost to the pig, and more research is needed to know whether they are a good marker of resilience.

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. Monitoring *Mycoplasma hyopneumoniae* infection alone would not have provided an accurate measure of disease severity or recovery in any of the above studies. However, resilience and recovery from disease may be better monitored by antibodies and anti-inflammatory immune responses that indicate the pig's immune system is fighting the infection challenge and recovering from disease.

Measuring the dam's impact on progeny health and disease

Pigs are born immunologically naïve and are dependent on immune components provided in the dam's colostrum and milk. These components are critical for passive immunity and protection from environmental pathogens in the naïve piglet. Passively transferred antibodies act with antimicrobial compounds (defensins, lectins, C-reactive proteins, and complement) from the sow's colostrum to detect and neutralise invasive pathogens prior to ingestion by phagocytic cells (Salmon *et al.*, 2009). Dams are able to transfer antibodies to the same pathogens that they have been exposed to, hence the value of vaccinating dams during late gestation against pathogens that newborns are likely to be exposed to. The adequate uptake of colostrum within the first 24 to 48 hours is vital for the piglet's survival and protection from disease. Piglet serum IgG concentrations can be used to measure the uptake and distribution of colostrum between litter mates. However, within most litters there is considerable variation in passive IgG uptake by piglets, and this is exacerbated with increasing litter sizes (Jourquin *et al.*, 2010). Early piglet health can also be assessed by measuring innate immune responses (acute phase proteins such as haptoglobin), pathogen specific antibodies or haemoglobin concentrations in the first days of life. Vaccinating sows in gestation will increase passive immunity in the newborn pig, but will not affect the development of the piglet's own adaptive immune response, so is only a short term measure for resilience.

Differences in the health, survival and growth of gilt and sow progeny in the first few weeks of life indicates that dam parity, as well as individual differences between dams, may influence pathogen exposure and immune response in the newborn piglet. Higher pathogen challenge was postulated as the reason for elevated concentrations of acute phase proteins (innate immunity) in piglets reared on gilts compared to older parity sows, regardless of the parity of their birth dam (Morales *et al.*, 2006). It is generally expected that older sows are better able to protect their piglets from infection because their colostrum and milk will be fortified with antibodies to the wider range of pathogens they have been exposed to, relative to younger gilts. Therefore measuring the concentration of specific antibodies in dam's milk may provide a measure of disease resistance, rather than resilience in their progeny. However, there is no clear

evidence that gilts are less able to transfer passive immunity to their progeny than sows. Sows or gilts vaccinated with a novel antigen were equally able to transfer passive immunity to their progeny, measured as specific antibody concentrations (Miller *et al.*, 2013). However, reduced antibody concentrations were observed in gilt-born progeny when they were vaccinated post weaning, suggesting that the parity of the birth dam may influence the adaptive rather than the passive immune response.

Measuring intestinal health

Maintenance of intestinal health and prevention of outbreaks of enteric disease are dependent on the bacteria present in the pig's intestine, but also on interactions between these bacteria and the cells lining the intestine, along with the pig's immune system and nutrients provided in the diet (Yu *et al.*, 2012). Intestinal health is optimal when these various factors complement each other in balance. Alterations in this balance have the potential to result in enteric diseases in the host. Major changes occur in all of these systems in this first 8 weeks of the pig's life, which coincides with significant disease challenges to the piglet both before and after weaning. Issues with intestinal health in pigs, such as severe diarrhoea or scouring, generally occurs during the first two weeks of life or in the two weeks following weaning (Wada *et al.*, 1996).

Commensal bacteria are normal members of the intestinal microflora and benefit the host by supporting development of the intestine, digestion, overall health and by preventing the colonisation of pathogenic bacteria (Brestoff and Artis, 2013). Colonisation of the porcine gut with beneficial bacteria early in life can protect the gut from invasion by pathogens such as *Escherichia coli* (Roselli *et al.*, 2007; Chapman *et al.*, 2009). The commensal microbial community deters the colonisation of pathogenic bacteria in the gastrointestinal tract by providing competition for resources and receptors on the epithelial cell surfaces, and by synthesising or inducing the synthesis of antimicrobial substances including organic acids and bacteriocins (Candela *et al.*, 2008; Jankowska *et al.*, 2008; Lu *et al.*, 2009).

Both commensal and pathogenic bacteria that colonise the piglet's intestine are initially transferred from the sow and the environment shortly after birth. Newborn piglets are colonised by the microflora in their immediate environment, including the dam's skin, mammary glands, vagina and faeces (Pedersen *et al.*, 1992; Mandar and Mikelsaar, 1996; Lindberge *et al.*, 2004; Gueimonde *et al.*, 2006). *Lactobacillus* species are an important bacterial group in early colonisation due to the piglet's need to catabolise lactose in milk and to prevent establishment of pathogenic bacteria within the gastrointestinal tract. *Lactobacillus* species are detected in healthy newborn piglet faeces within four hours of birth, whilst coliforms, such as *Escherichia coli* are detected after eight hours (Muralidhara *et al.*, 1977). Within 24 hours of birth, *Lactobacillus* species and coliforms are present at nearly equal levels.

The health of the dam, along with hygiene in the farrowing crate therefore plays a critical role in the transmission of both beneficial commensal bacteria and potential pathogens to the newborn pig. Measuring the microbial balance in the sow's intestine may provide a method to

estimate protection of the piglets from disease. Once the mix of bacteria in the intestine (microflora) becomes established, the intestine is relatively resistant to significant changes in bacterial populations. However, dietary changes (including weaning), pathogen challenge or antibiotics all have the ability to cause dysbiosis in the intestinal microflora.

The identification of certain bacterial species that inhibit or reduce pathogen infection may also be a valuable tool in monitoring gut health, resistance and resilience to disease in the live pig. Competitive inhibition or exclusion by favourable bacterial species is the means by which probiotics and prebiotics work. The bacterial population of the gut is modified by providing either bacteria or nutrients that confer a competitive advantage for one bacterial species over another. Monitoring numbers or ratios of commensal to pathogenic bacteria or microbial diversity may provide a good estimate of the weaner pig's intestinal health and resistance to pathogen attack. Ten-fold higher ratios of Lactobacilli to *Escherichia coli* were observed in healthy weaner pigs relative to scouring weaners (Collins and Bowring, 2014). Likewise ratios of Lactobacilli to *Clostridium perfringens* (cause of diarrhoea in piglets) were ten-fold higher in healthy pigs relative to scouring pigs. Conversely, higher ratios of *Escherichia coli* to Enterobacteriaceae observed in scouring pigs relative to healthy pigs indicate overgrowth of *Escherichia coli* and intestinal disease (Collins and Bowring, 2014). Higher levels of some commensal bacteria prior to Salmonella challenge are associated with reduced Salmonella shedding later (Bearson *et al.*, 2013), suggesting that the intestinal microflora has a significant impact on disease expression and recovery.

Ratios of commensal to pathogenic bacteria need to be interpreted with care because broad spectrum antibiotics can destroy both commensal and pathogenic bacteria. Studies have shown that pigs medicated with antibiotics have decreased numbers of commensals (Lactobacilli, *Streptococcus* and *Bacillus* species) and increased numbers of pathogenic bacteria (*Escherichia coli*) (Collier *et al.*, 2003; Looft *et al.*, 2012). With recovery from disease, it is expected that *Escherichia coli* numbers decrease and Lactobacilli numbers increase, leading to an overall increase in this ratio. However, these ratios need to be correlated with disease measures to ensure that Lactobacilli numbers are reduced because of disease and not just because of antibiotic medication. Resilience could therefore be measured as the time required to clear infection and restore the balance to the microbial flora.

Samples for monitoring herd health and immunity

Pig disease and immunity measured at the herd level requires an appropriate sampling protocol that takes into account the expected prevalence of disease to estimate the number of samples required to accurately estimate the pathogen load or level of immunity in the herd (Cannon and Roe, 1982). The detection of antibodies in serum samples is a cost-effective way to determine the prevalence of infection, proving previous infection. In most cases, once circulating antibodies are detected, pigs have recovered from infection and are immune, so antibodies are a good measure of recovery, but in adaptive immunity it is not clear if antibody level correlates with resilience, although antibodies show high heritability (Flori *et al.*, 2011).

Pooling of samples has also been investigated as a means to reduce laboratory costs, but it needs to be remembered that pooling samples can reduce the sensitivity of the assay. This is especially important when dealing with low prevalence and sub-clinical disease. A quantitative PCR for *Lawsonia intracellularis* was able to detect one positive animal in a pool of ten negative animals, when the positive sample was from a clinically affected pig. However, at least 6 of 10 samples had to be positive in sub-clinically affected pigs (Collins and Barchia, 2013). Likewise, five was the maximum number of faecal samples that could be pooled to provide an accurate estimate of the number of *Lawsonia intracellularis* excreted by pigs. Pooling ten samples provided a poor representative of *Lawsonia intracellularis* numbers detected by a quantitative PCR relative to the same samples tested individually (Collins and Barchia, 2013).

Oral fluid sampling monitors pig health in groups of pigs within a population, rather than sampling a relatively large number of individual pigs within a population. Oral fluids can contain both pathogens and the immune response to these pathogens (Prickett *et al.*, 2008). 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. In field studies, qPCR detection of PCV2 was more sensitive in single oral fluid samples than in blood samples collected from a subsample of animals. However, the sensitivity of detecting PCV2 antibodies in the same blood samples was more sensitive than the oral fluid samples. A significant correlation was also observed between the mean *Lawsonia intracellularis* antibody concentration in serum and the mean load of *Lawsonia intracellularis* in oral fluids (Finlaison and Collins, 2014).

Summary

The pig's ability to recover from disease is based on host, pathogen and environmental factors, including pathogen numbers, their virulence, the pig's genetics and immune responses and environmental factors such as air quality, temperature and humidity. The outcome of an infection depends upon resilience in the host as well as an ability to resist and tolerate infection. Recovery from disease is assisted by treatment options such as antibiotics, probiotics, prebiotics, improved hygiene and vaccination. However, it is largely the host's immune system that dictates the rate and extent of recovery. 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. An animal's ability to exert control over a pathogen or resist infection can be measured by immune responses, but also by pathogen load and competitive inhibition by other bacterial colonisers at the site of infection. Measuring antibody titres, pro- or anti-inflammatory cytokines, acute phase proteins, pathogen load and ratios of commensal to pathogenic bacteria will help determine a host's ability to resist a pathogen, but the impact of the environment will also affect recovery from infection. Monitoring the negative feedback mechanism required to dampen the inflammatory response may equate more closely with resilience. However more research is required to understand the relationship between immune

responses, the repair of damaged tissues, the resumption of normal metabolic processes and pig growth.

Monitoring feed intake, weight gain and feed conversion ratio will certainly demonstrate the pig's return to good health and are technically a measure of performance outcomes following infection, but they provide no information about the host's interaction with the causative pathogen, and what the infection has cost the host. Measures of health, infection, immune stimulation, disease severity and recovery need to be correlated to feed intake and growth in order to quantify tolerance in pigs. Where weight gain correlates well with specific pathogen numbers, quantifying the pathogen load over time can demonstrate those pigs that recover more quickly and hence are tolerant. This is more difficult to interpret when multiple pathogens are involved and when genetic and environmental factors exacerbate disease. Similar relationships need to be identified between immune and production parameters to increase the usefulness of immune markers to measure resilience and recovery from disease.

The pig's ability to resist disease also depends on the pig's health at the time of infection. The health of pigs has been measured by quantifying commensal or beneficial bacterial populations at the site of infection, as well as protection afforded by the passive transfer of antibodies to piglets via milk and colostrum. However, the process of weaning has significant impacts on pig health in both of these measures. The change from a predominantly milk to a grain diet at weaning affects the microbial balance in the gut and also concludes the transfer of passive protection from the dam. Modifying weaner diets to reduce protein levels can reduce the severity of scouring caused by *Escherichia coli* infection (Kim *et al.*, 2011) but more research is needed on the effect of other dietary supplements to reduce the incidence of disease or stimulate a more effective immune response.

Acknowledgements

The author acknowledges the financial support of NSW Department of Primary Industries (DPI) and the Australian Pork CRC for High Integrity Pork for many years of herd health monitoring research. The support of colleagues, research students and technical officers at both NSW DPI and the University of Sydney has been integral to the provision of background data for this review.

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