Project 2B-106: Simple tests for immune responsiveness of sires and the association with piglet mortality

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

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Executive Summary

Selection for improved health and disease resistance attributes is generally difficult in breeding operations due to a lack of cost-effective suitable measures which can be applied in the field, combined with high health status and extensive vaccination schedules of nucleus herds, which limit opportunities to develop selection criteria using a standardised natural disease challenge. An alternative approach for animal breeding applications is to assess immune competence in selection candidates using a measureable phenotype (Hine *et al*. 2012). Such phenotypes are moderately heritable (Wilkie and Mallard, 1999) and have been shown to be associated with a range of health related traits. For example, pigs selected for high immune competence had consistently higher response to commercial vaccines, fewer mummified fetuses following a natural outbreak of parvovirus infection, lower overall disease scores following challenge with *Mycobacteria hyorhinus*, and improved rate of gain compared to pigs of the low or control lines (Magnusson *et al*. 1999; Wilkie and Mallard 1999; 2000). In this study, we wished to re-establish proof of concept for a commercial breeding strategy which had achieved no traction in pig breeding herds to date, but which is now widely used in the dairy industry (Mallard *et al*. 2014).

The study involved two parts. The first part (a pilot project) was used to establish a commercially viable protocol for measuring immune competence of mature boars, which is potentially complicated by their long vaccination history. The second part (the main project) involved obtaining immune competence phenotypes for mature boars, after vaccination with a novel multi-valent vaccine (Ultravac® 5in1). This vaccine was used to induce a measureable antibody response (AMIR) to *C. tetani* toxoid, along with a cell-mediated response (CMIR) measured as a delayed type hypersensitivity skin test. The AMIR and CMIR phenotypes were then used to assign boars to nine immune competence groups by combining tertile groups assigned separately for the AMIR and CMIR traits. Immune group was then tested as an explanatory variable for differences amongst sires in estimated breeding values for a range of traits, including piglet mortality and later performance traits. The underlying assumption was that piglet mortality phenotypes, when corrected for other known factors which contribute to mortality, are then likely to represent the impact of differences amongst piglets in their health and immune competence.

Results from the main study demonstrated variability amongst boars (N=87) in their own phenotypes for both AMIR and CMIR. Approximately 28000 progeny of these boars were subsequently recorded for individual mortality outcomes. Immune group was established as a significant ($p<0.0001$) factor associated with EBVs for pre-weaning mortality of progeny, but no other economically important performance trait. Therefore, on the basis of results from this and previous studies, we recommend further research on measuring immune competence in nucleus herds for pigs, with the aims of demonstrating 1) inheritance of these immune competence measures; 2) potential benefits of enhanced immune competence (eg. improved efficacy of vaccination or health status, reduced medication costs, improved animal welfare etc), and 3) estimating genetic parameters and developing appropriate breeding strategies to improve health and welfare through indirect selection for immune competence.

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1. Introduction

Selection for immune competence is difficult generally due to a lack of cost-effective and suitable measures which can be applied in the field, combined with the frequently high health status of nucleus herds and extensive vaccination schedules, which limit opportunities to develop selection criteria using a standardised natural disease challenge. However, immune competence is generally important in all production species, particularly in commercial environments. Selection for productivity with little or no emphasis on health and fitness traits can have undesirable consequences for health and immunological traits in many food producing animals (Rauw *et al*. 1998). Immune competence influences overall herd health, reproductive performance, performance in production traits along with survival (Mallard *et al*. 2014; Mallard *et al*. 2015), and also has an impact on the efficacy of vaccination programs (Wilkie and Mallard, 1999).

An alternative approach for animal breeding is to assess immune competence with a measureable phenotype (Hine *et al*. 2012). The strategy for assessing immune competence proposed for this project is based upon the University of Guelph High Immune Response (HIR) technology. This approach was originally researched in pigs in the 90's, and is now routinely used in the Canadian Dairy Industry (see https://www.youtube.com/watch?v=l0FRsDKgMs0). Testing to identify HIR animals is currently under investigation in Australia for beef cattle, particularly for feedlot applications. The HIR testing strategy is targeted at identifying animals with optimized or balanced immune responsiveness, which is associated with enhanced resistance to a broad range of diseases – not just to the antigen(s) used to test the immune responsiveness.

The procedure to identify HIR animals tests for two components of immune responsiveness: humoral and cell-mediated immune responses. Humoral response is assessed by measuring antibody production in response to vaccination, while cell-mediated immune response is assessed by measuring the magnitude of delayed type hypersensitivity reactions to vaccine components using a skin test. Measures of these components of immune responsiveness are then combined to evaluate individual animals for their overall immune competence.

The delayed type- hypersensitivity test (DTH) conducted as part of testing is a standard method used extensively in the past for detecting bovine tuberculosis (TB) in cattle. The procedure is relatively simple to conduct and generates reliable test data on-farm within 48 hours. DTH reactions to a variety of antigens have been assessed previously (i.e. not just TB). Alternative laboratory based techniques to detect the magnitude of cell-mediated immune responses include quantification of IFN-γ production following stimulation of cells using ELISA techniques, and lymphocyte proliferation assay. However, these procedures are generally considered too limiting for large scale testing due to both cost and logistics as they require the use of freshly collected samples, which is why DTH response is the preferred measurement for field testing of cell-mediated responses. The traits used for HIR testing in both pigs and cattle have previously been demonstrated to be moderately heritable (Wilkie and Mallard, 1999).

To date, the potential of immune response testing has not been considered within the Australian pig Industry, despite pigs in commercial piggeries encountering numerous health challenges. After a pilot project to establish a suitable methodology specifically for assessing mature sires, this project will characterise sires for their immune responsiveness using a relatively simple standardised procedure. Then, assuming that differences amongst sires in their immune responsiveness will be inherited by their progeny, and that immune responsiveness will have an impact generally on mortality outcomes in a commercial herd, we will then quantify the association between general measures of immune responsiveness and progeny mortality using extensive data on pre- and post-weaning mortality of piglets. This will provide proof of concept that variation in immune responsiveness of sires is reflected in their progeny survival outcomes.

Immune response testing procedures are expected to be relatively easily adapted to practical breeding program applications, because with appropriate consideration it could be incorporated into existing management practices, such as routine vaccination post-selection, and requires less laboratory testing, since the data for cell-mediated immunity is derived from relatively simple field observations. The power of BLUP genetic evaluation systems is such that not all animals require testing for all traits, and therefore opportunities exist to target immune response testing to improve cost-effectiveness (i.e. measuring only some selection candidates) while providing improved opportunities for selection overall.

Improved immune competence obtained through selection in breeding herds is cumulative and disseminated throughout commercial herds, and should have measureable impact on survival, herd health and performance traits. In previous studies, high immune response, low immune response and randomly bred control lines were established over 9 generations of selection and pigs examined for health and performance. High line pigs had consistently higher responses to commercial vaccines, fewer mummified fetuses following a natural outbreak of parvovirus infection, lower overall disease scores following challenge with *Mycobacteria hyorhinus*, and improved rate of gain compared to pigs of the low or control lines (Magnusson *et al*. 1999; Wilkie and Mallard 1999; 2000). Therefore, there is prior evidence that suggests that selection for improved immune responsiveness will improve the health, welfare and productivity of pigs.

In a more recent example of the commercial impact in dairy cattle, health data was collected on progeny from high immune responder animals (Immunity+) versus progeny from sires of unknown immune responsiveness status (see Table 1). Clear reductions in disease occurrence were evident for daughters of Immunity+ sires, whose immune competence was established using similar procedures as described above.

Table 1. Disease Occurrence of Immunity+ Daughters in a Large US Dairy Herd in 2013 (Data Courtesy of Jay Shannon, Semex Alliance)

Disease	Cattle	Immunity+ Daughters	All Other Daughters	Disease Reduction
Mastitis	1st Lact.	8.8%	15.8%	44.3%
All Recorded Disease	1st Lact.	16.7%	18.2%	8.5%
Pneumonia	Heifers	6.8%	9.1%	25.3%

(From Mallard et al., 2014)

Testing strategies suitable for commercial breeding populations in pigs, where restrictions exist on the use of unregistered products, have not yet been established. The aim of this project was to develop a testing procedure suitable for assessing mature boars to identify those with superior immune competence, and to subsequently investigate if sire variation in immune competence measures was reflected by differences in the survival of their offspring (pre- and post-weaning), and/or potentially other performance attributes.

2. Methodology

Pilot study

A pilot project was conducted to establish both a suitable antigen and injection site for generating measurable delayed type hypersensitivity (DTH) response in mature boars with an extensive *a-priori* vaccination history. It was important that the antigen(s) used were commercially acceptable for use with pigs (i.e. they cannot be antigens unregistered for use in animals which may enter the food chain). A second requirement was that mature boars had not previously been exposed to the antigen(s), either by pre-existing vaccination policies or a high probability of natural infection. Finally, the chosen antigen(s) needed to induce a reliable, measureable response to permit both cell-mediated (CMIR) and antibody-mediated (AMIR) immune responses to be assessed. After examination of vaccination schedules and endemic disease status, tetanus toxoid was chosen as the model antigen for this study.

Measurable antibody responses to tetanus toxoid following vaccination have been reported in many farm animals, but previous laboratory results in pigs had suggested that vaccination with *C. tetani* alone was not a good model to assess cell-mediated immune response (Miller *et al.* 2008). Conversely, an excellent DTH response has been observed using a 5in1 vaccine in cattle (i.e. a vaccine containing 5 clostridial antigens). DTH responses to 5in1 vaccine likely represent a combination of responses to the various components of the multi-valent vaccine (including *C. tetani*), and some contribution from innate responses to the classical DTH responses measured during testing are expected when using a multi-valent vaccine (Brad Hine, personal communication). The DTH response (rather than laboratory tests) was the preferred field measurement to assess cell-mediated immunity in this study. Therefore we compared intradermal antigen response following vaccination with a multivalent vaccine.

Previous work had also demonstrated that DTH responses increase skin-fold thickness by double, on average. Therefore, 10 boars were sufficient for a pilot study aimed at establishing whether a significant DTH response could be observed using the testing procedure. On day zero (D0) of the study, 10 boars were bled to assess baseline antibody levels to tetanus toxoid and were then vaccinated with Ultravac® 5in1 (Zoetis Animal Health), which contains the tetanus toxoid antigen along with 4 other clostridial antigens and an adjuvant. A booster vaccination was delivered on D21. On D30, each boar received intradermal injections of saline and either Equivac® T (Zoetis Animal Health) or Ultravac® 5in1 (both containing the tetanus toxoid antigen) at 3 potentially suitable injection sites (ear flap, ear base and perineal area). The magnitude of DTH responses was assessed by measuring the change in skin thickness between D30 and D32 or D33, and this measure was used to compare the suitability of the alternative antigens, sites and timing of recording to obtain a phenotype for the DTH response. A summary of the timing of activities for the pilot study is provided in Table 2.

Antibody testing for tetanus toxoid was conducted using blood samples collected on D0 (pre-vaccination), D14 (primary response) and D30 (secondary response). The antibody response was represented as a sample to positive ratio, from an in-house assay developed using tetanus toxoid antigen provided by Zoetis (Miller *et al*. 2008). Commercial assays have previously been shown to vary in their accuracy for determining tetanus toxoid IgG antibodies (Perry *et al*. 2009) and the phenotype represents a relative response rather than an absolute titre, which was not required in our study. AMIR phenotypes were therefore calculated as a sample to positive ratio.

Table 2. Schedule of activities required to obtain phenotypes for AMIR (blue) and CMIR (green*) measures of mature boars in a pilot study

Day	Activity	Time
D0	Baseline bleed for tetanus toxoid antigen antibodies Ultravac® 5in1 vaccination	-
D14	Blood sample to measure primary response to tetanus toxoid antigen	-
D21	Ultravac® 5in1 booster vaccination	-
D30	Blood sample to measure secondary response to tetanus toxoid antigen Baseline double skin thickness measured with Harpenden skinfold caliper (ear base, ear flap, perineal area) Intradermal injection of Ultravac® 5in1 or Equivac® T + saline control (ear base, ear flap, perineal area)	T0h
D32	Double skin thickness measured with Harpenden skinfold caliper (ear base, ear flap, perineal area)	T48h
D33	Double skin thickness measured with Harpenden skinfold caliper (ear base, ear flap, perineal area)	T72h

*the DTH response as an indicator of CMIR is conditional on the prior vaccination activities

Main study

Based on results from the pilot study, test procedures were simplified as below (Table 3) for the main study.

Table 3. Schedule of activities required to obtain phenotypes for AMIR (blue) and CMIR (green*) measures of mature boars in the main study

Day	Activity	Time
D0	Baseline bleed for tetanus toxoid antigen antibodies Ultravac® 5in1 vaccination	-
D21	Ultravac® 5in1 booster vaccination	-
D30	Blood sample to measure secondary response to tetanus toxoid antigen Baseline double skin thickness measured with Harpenden skinfold caliper (perineal site) Intradermal injection of Equivac® T + saline control (perineal site)	T0h
D32	Double skin thickness measured with Harpenden skinfold caliper (perineal site)	T48h

Subsequently, approximately 100 boars in the Woodlands Centre at Rivalea (representing three selection lines) were tested to determine their individual AMIR and CMIR phenotypes. These boars had large numbers of pedigreed progeny (and ancestors) recorded for a range of traits, including mortality pre- and post-weaning, as well as production and reproductive traits (maternal lines only). Therefore, each boar had an estimated breeding value for a range of traits recorded on either/or themselves, their ancestors and their progeny.

The phenotypes for immune competence (AMIR and CMIR) were calculated as follows:

$$\text{AMIR phenotype: } \frac{\text{(Sample absorbance at Day 30 - Negative control mean}^{A})}{\text{(Positive control mean}^{B} - \text{Negative control mean}^{A})}$$

where: means were of the three lowest (^{A}) and highest (^{B}) sample absorbance values on Day 0 or Day 30 (within plate).

$$\text{CMIR phenotype: } (\text{Antigen}_{32} - \text{Antigen}_{30}) - (\text{Saline}_{32} - \text{Saline}_{30})$$

where: Antigen (Saline) refers to the double skin thickness recorded at the antigen (saline) injection sites on days 30 and 32.

The association between immune competence phenotypes of sires with their breeding values for a range of other traits was subsequently investigated by characterising boars into defined immune competence groups, and testing whether this immune grouping was significantly associated with breeding values, accounting for line in the model.

3. Outcomes

Pilot study

There was a significant difference between sites in the DTH response observed (p=0.0001), but there was no significant difference (p=0.164) between Ultravac® 5in1 or Equivac® T as the source of antigen to stimulate a DTH response. The ear flap was confirmed to be an easily accessible but inappropriate site to measure a DTH response, probably due to physical constraint of skin swelling by the underlying cartilage of the ear. The maximum DTH response measured on the earflap was relatively low (23%, Table 4) compared to the other sites. The DTH response in the perineal area was more variable than at the base of the ear, but more accessible and could be better standardised with further training. There was also no significant change in the DTH phenotype from skin measurements taken at 48 compared to 72 hours post the intradermal injection on D30 (Table 4). Therefore, skin measurements taken 48 hours after the intradermal injection would appear sufficient (Table 4).

Table 4. Least squares means for skin thickness (mm) by treatment (saline vs antigen), time (T0, 48h, 72h) and site (Ear flap, Ear base, Perineal area), averaged over intradermal antigens

	Time		
Treatment	T0	48h	72h
Ear flap			
saline	4.90±0.18	4.91±0.18	5.02±0.18
antigen	4.79±0.44	5.15±0.44	5.12±0.44
Antigen-saline	-0.11±0.43	0.24±0.43	0.09±0.43
DiffT0 (mm)	0	0.38±0.42	1.09±0.42
DiffT0 (%)	0	8.25±11.15	23.0±11.2
Base of ear			
saline	3.35±0.18	3.81±0.18	3.59±0.18
antigen	3.08±0.44	6.16±0.44	5.52±0.44
Antigen-saline	-0.28±0.43	2.34±0.43	1.92±0.43
DiffT0 (mm)	0	3.06±0.42	2.42±0.42
DiffT0 (%)	0	95.9±11.2	77.8±11.2
Perineal area			
saline	3.29±0.18	4.44±0.18	4.17±0.80
antigen	4.12±0.44	7.10±0.44	8.01±2.08
Antigen-saline	0.84±0.43	2.67±0.43	3.84±2.33
DiffT0 (mm)	0	2.96±0.42	3.90±1.99
DiffT0 (%)	0	79.5±11.2	97.4±11.2

Boars also showed variable antibody responses to the vaccination protocol. The SP ratio of the secondary response was relatively higher than the SP ratio from the primary response (data not presented). This preliminary study demonstrated that boars differed in their AMIR and CMIR phenotypes, as illustrated in Figures 1a and 1b.

Figure 1a. Sample to positive ratio of pilot study boars assessed at D30 after the initial (+ booster) vaccination

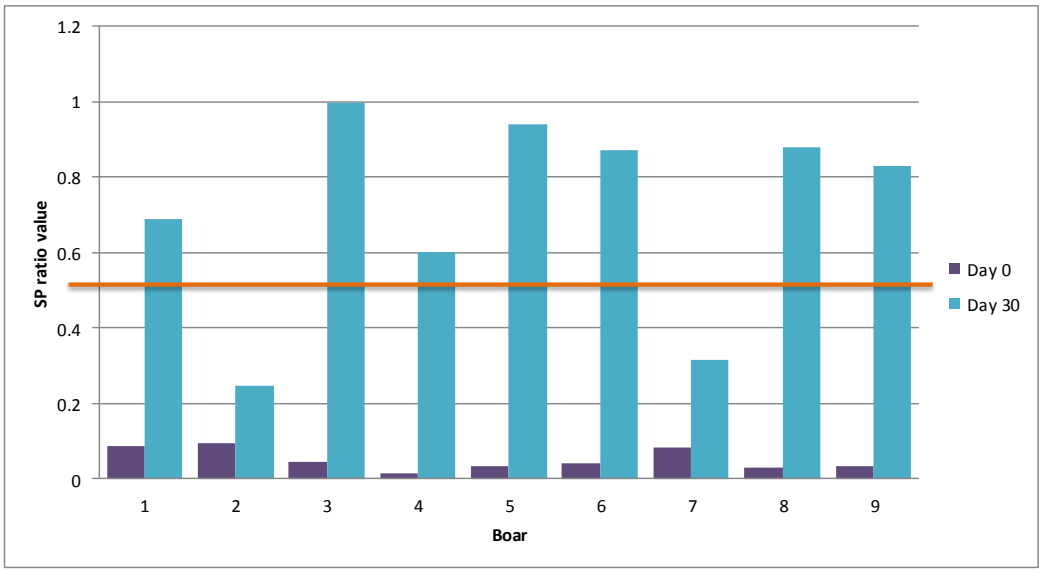
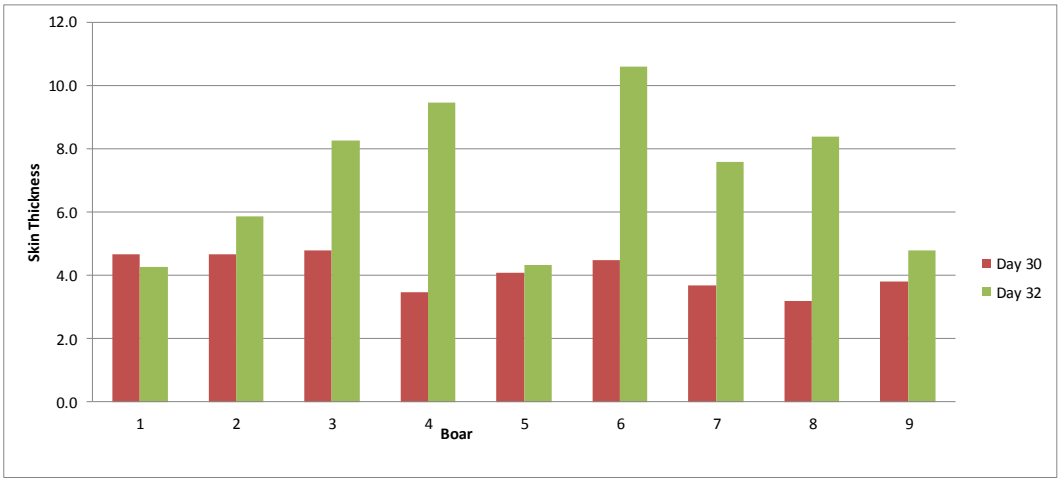


Figure 1b. Double skin thickness (mm) of pilot study boars prior to intradermal injection of an antigen (D30) and two days later (D32)



Overall, results from the pilot study demonstrated that vaccination with a commercial multivalent vaccine, Ultravac 5in1®, resulted in a measureable anti-*C. tetani* antibody response, and a measurable cell-mediated response as assessed by measuring magnitude of DTH responses following intradermal injection of either Ultravac® 5in1 or Equivac® T. Therefore it is possible to implement simple testing procedures to assess immune competence of sires in Australian breeding programs. Ideally such measurements would be adapted for recording in much younger selection candidates, because this would provide new selection criteria prior to their selection.

Results from this pilot study were published in the following paper:

Harper J, Bunter KL, Hermesch S, Hine BC and Collins AM (2017). Modifying procedures to assess immune competence in mature boars. *Animal Production Science* **57**: 2464 (APSA conference proceedings).

Main study

Breed grouping of boar (maternal vs terminal lines) significantly (p=0.037) affected CMIR but not AMIR (p>0.05). Sires from the terminal line had higher CMIR. Therefore, ranking individual boars into tertiles for AMIR and CMIR traits was conducted within each of Maternal vs Terminal breed grouping. Group 1 represents the lowest tertile, group 2 is the middle tertile and group 3 is the highest tertile. Characteristics of immune phenotypes for these immune groups are shown in Table 5.

Table 5. The distribution of boars across tertiles for AMIR and CMIR separately, along with mean values (SD) by breed group

	Maternal (N=54)				Terminal (N=33)			
Group	AMIR		CMIR		AMIR		CMIR	
	N	Mean(SD)	N	Mean(SD)	N	Mean(SD)	N	Mean(SD)
1	18	0.29 (0.10)	17	0.90 (0.66)	11	0.28 (0.14)	11	0.71 (0.96)
2	18	0.50 (0.06)	19	2.33 (0.38)	11	0.53 (0.05)	11	3.27 (0.58)
3	18	0.78 (0.13)	18	3.69 (0.61)	11	0.82 (0.17)	11	5.28 (1.03)

The breakdown of animals across the combined AMIR and CMIR tertile groups (i.e. hereafter termed immune group) is shown in Table 6. This immune grouping is represented hereafter as the combined codes (eg. immune group 11 is the group of boars with AMIR group=1 and CMIR group=1). The Pearson correlation between the separate group allocations for AMIR and CMIR was 0.31 (p=0.003), suggesting that animals with low AMIR were less likely to have high CMIR. Therefore, there were fewer animals in the immune group combinations representing opposing extremes (ie. 13 or 31) than there were in groups showing similar ranking for both AMIR and CMIR (ie. 11, 22 and 33). Across both breed groups, in total 43-45% of all boars fell into the three main group combinations (11, 22 & 33).

Table 6. Distribution of boars across combined groups (Igroup) for AMIR (rows) and CMIR (columns)

	Maternal (N=54)			Terminal (N=33)		
Group	1	2	3	1	2	3
1	8	6	4	4	5	2
2	7	6	5	5	4	2
3	2	7	9	2	2	7

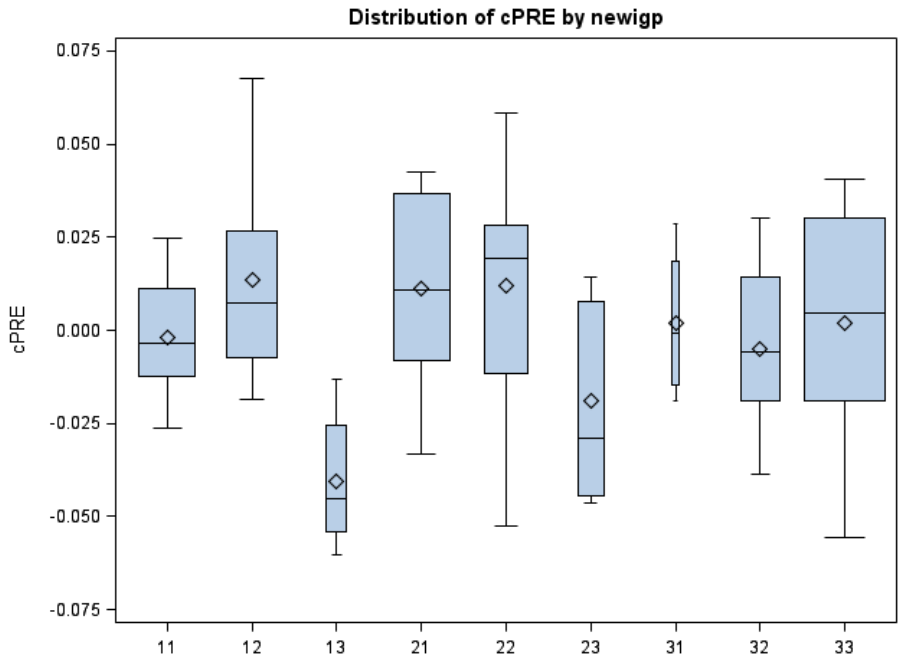
The significance of grouping based on combined AMIR and CMIR (Igroup) for differences in genetic merit estimated independently for a range of other economically important traits is shown in Table 7. Breeding values significantly differed by line, as expected, because lines have different levels of emphasis placed on the range of traits recorded at selection. Moreover, the breeding values for each line are generally obtained from independent analyses. Therefore, mean EBV can differ by line due to historical selection emphasis, line specific analyses used to obtain the breeding values, and the sample of boars available per line. After accounting for line, immune grouping was not a significant factor explaining variation amongst boars in EBVs for any trait except pre-weaning mortality (PRE).

Pre-weaning mortality breeding values for boars in the main study were estimated using more than 420K individual piglet records recorded over 9 generations, including progeny of these boars. At the time of analysis, a total of ~28000 progeny contributed to their estimates of EBVs for mortality. Comparing smaller subsets of boars with higher accuracy of breeding values did not alter the conclusions which can be drawn from this study (data not presented). The model to estimate PRE EBVs accounted for litter size, gestation length and birth weight, dam factors such as parity, fostering events (across lines) as well as breed and contemporary group effects fitted as systematic effects. Random effects included birth and nurse litter along with repeated performances for the nurse sow, established after fostering on D1. Therefore, the model to predict EBVs for boars accounted for many of the other known key contributors to individual piglet mortality outcomes, with the exception of immune status.

The only contribution a boar can make to their progeny survival is the transmission of genes. Our results suggest that in addition to the known significant factors included in the genetic evaluation model for PRE, inherited genes related to the observed immune competence phenotypes of boars had a measureable impact on pre-weaning survival, as reflected by the EBVs for PRE. High CMIR (group 3) response was particularly important when AMIR response was low (AMIR groups 1 & 2). The best (lowest) EBVs for pre-weaning mortality occurred for boars in Igroups 13 (6 boars) and 23 (7 boars).

The distribution of EBVs for boars allocated to immune groups is shown in Figure 2. EBVs of boars for pre-weaning mortality over-lapped across immune groups, as would be expected. However, the average superiority of boars in groups 13 and 23 for PRE was evident despite the relatively small sample of boars in each group.

Figure 2. A boxplot to illustrate the distribution of breeding values for pre-weaning mortality, corrected for line (cPRE), by immune grouping*



*width of the box is proportional to the number of boars in the group

Results from the main study have been submitted for publication in the following conference paper:

Harper J., Bunter K.L., Hine B.C., Hermesch S. and Collins A.M. (2018). The association between measures of immune competence of boars and survival of their purebred progeny

Table 7. Least squares means for trait EBVs by immune group, after accounting for sire line. The number of sires and the total number of their progeny included in mortality analyses at the time of reporting is shown.

Trait Igroup[A]	Sires	Progeny	ADG	BF	FCR	IGF	BWT	PRE	POST	MAT	NBA*	TNL*	TEAT*
11	11	3147	95.3	-1.55	-0.29	-23.9	32.5	-0.038[b]	-0.002	-0.025	2.49	0.72	0.72
12	11	3504	102	-1.75	-0.29	-26.3	50.0	-0.022[b]	-0.013	-0.008	2.77	0.75	0.41
13	6	2468	69.3	-1.59	-0.33	-26.2	38.6	-0.078[a]	0.000	-0.027	2.40	0.62	0.30
21	12	3281	96.4	-1.43	-0.27	-22.7	23.9	-0.025[b]	-0.007	-0.006	2.24	0.71	0.60
22	10	3763	111	-1.21	-0.30	-23.4	7.9	-0.025[b]	-0.010	-0.013	2.27	0.58	0.54
23	7	1947	104	-1.15	-0.31	-25.8	-8.1	-0.054[a]	-0.018	-0.027	2.42	0.75	0.32
31	4	1483	86.9	-1.37	-0.24	-23.1	-0.1	-0.033[ab]	-0.011	-0.025	2.42	0.63	0.66
32	9	3041	89.0	-1.27	-0.28	-23.7	26.8	-0.041[ab]	-0.011	-0.019	2.57	0.58	0.44
33	6	5436	93.4	-1.52	-0.29	-23.8	43.2	-0.034[b]	-0.010	-0.014	2.40	0.64	0.49
			Significance (p-values) for Line and Igroup, along with model R^2										
Line			0.0002	<0.0001	<0.0001	<0.0001	0.0008	<0.0001	<0.0001	0.001	0.56	0.52	0.03
Igroup			0.23	0.94	0.42	0.94	0.68	0.004	0.37	0.17	0.63	0.68	0.66
Model R^2 (%)			28.1	64.3	88.5	80.8	23.3	41.5	39.7	28.2	12.3	11.5	21.6

[A]The Igroup code is the combined codes from independent AMIR and CMIR groups (eg. Igroup 11: AMIR group=1 and CMIR group=1)

*EBVs for reproductive traits (NBA, TNL, TEAT) available for N=54 maternal line boars only

ADG: average daily gain (g/day): BF: back fat (mm); FCR: feed conversion ratio (kg/kg); IGF: juvenile IGF-I (µg/ml); BWT: piglet birth weight (g); PRE: pre-weaning mortality (0/1); POST: post-weaning mortality (0/1); MAT: maternal pre-weaning mortality (0/1); NBA: number born alive (pigs/litter); TNL: longevity (parities); TEAT: count of teats.

Pairwise comparisons with different superscripts are significantly different from each other.

4. Application of Research

In this study, we developed a modified immune responsiveness testing procedure suitable for use in mature boars to estimate their own immune competence phenotype, and were subsequently able to demonstrate that variation between these boars in their immune competence phenotypes was associated with differences in their EBVs for pre-weaning mortality, which were derived from large numbers of progeny and historical data. Since the only direct impact sires can have on their progeny performance is through the transmission of genes between sire and offspring, this suggests that there was a genetic component which influenced piglet survival, related to immunity, which was transmitted from sire to offspring. Therefore, our hypothesis that immune competence of boars would be important for survival outcomes of their progeny would appear to be supported.

From the relatively small sample of sires available in this study, the most diverse sire immune groups ranged in mean genetic merit for piglet pre-weaning mortality from -0.022 to -0.078. Half of this 5.6% difference would be inherited by offspring, giving an expected difference of 2.8% in progeny mortality. This is equivalent to an extra 28 pigs sold per 100 litters born. Even with no other obvious benefits for other traits, this type of difference in output is worth pursuing. However, previous studies in other species suggest that the gains from improving immune competence are multi-dimensional. For example, the introduction of immune testing in the dairy industry, intended primarily to reduce mastitis, resulted in HIR cows with "a 19-30% lower disease incidence compared to herd averages. These cows also respond better to commercial vaccines and produce higher quality colostrum" (See http://www.semex.com/tsa/i?viewnews=1439430315). These other types of benefits reduced costs and increased productivity over and above the gains observed solely from reducing incidence of mastitis. Similar studies should be performed for pigs in a larger scale study to more fully quantify the possible benefits; for example, increased efficacy of vaccination and the likely range in differences in mortality with a larger sample of sires.

The methodology described here is expected to be largely transferable to younger animals. This is important for at least two reasons: 1) selection candidates are young animals, and response to selection is generally improved if phenotypes are obtained on selection candidates prior to selection decisions; and 2) older (selected) boars are difficult to handle. Only 87 out of the original 100 boars retained for the project were able to be fully recorded for immune competence measures, largely due to OH & S issues. A further argument for assessing younger animals is that test procedures could potentially be modified to be complementary with already established vaccination schedules to induce measurable responses. The obvious counterargument is that this complementarity might not be possible because of endemic exposure to antigens frequently vaccinated against. One of the clear benefits of using an antigen model, such as vaccination against *C. tetani*, was that the model antigen(s) were novel because the current indoor environment and vaccination schedules minimised prior exposure to this organism. This might not be true, however, for outdoor environments.

Selecting for balanced immune competence is a generic approach (Hine *et al*. 2012) with potential for enabling improvements in pig health and welfare. It does not involve direct selection against specific disease(s) of interest, which is generally not feasible in nucleus herds. Preliminary results from this study suggest that there were associations between immune competence grouping and pre-weaning survival, and no antagonistic associations between the sire immune competence phenotype and other economically important traits. These preliminary results suggest that selecting for aspects of immune competence using a model antigen testing procedure is feasible in the Australian pig industry, and further work in this area is very desirable.

5. Conclusion

In this project we developed a test procedure to obtain phenotypes for assessing immune competence on mature boars. These procedures are feasible to introduce into commercial breeding programs, and provide an alternative approach to direct disease exposure as a means to select for improved health in pigs. Immune competence phenotypes of boars were significantly associated with estimates of EBVs for pre-weaning mortality. A larger study should be undertaken to validate the findings from this relatively small scale study, and to identify the range of possible benefits which could result from improved immune competence.

6. Limitations/Risks

The main limitation to this study lies with the relatively small sample of boars which were available for testing within the time frame available. Further work should be conducted to obtain more data on immune competence phenotypes, to establish genetic parameters and also the relationships between immune competence measures and other traits. Additionally, the contribution of improved immune competence to disease incidence, health care costs and productivity should be further quantified in a commercial environment.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- Progeny of sires included in the main study of this project should be tested for immune competence measures to establish some consistency in transmission of immune competence phenotype from sire to offspring
- Further investigation of testing for immune competence on disease incidence, health care costs and performance traits should be conducted using a larger scale study. Concurrent genotyping might be a useful addition to this work.

- The Australian pig industry should consider the potential benefits, direct and indirect, which could result from using immune competence phenotypes as selection criteria.

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