The association between measures of immune competence of boars and survival of their purebred progeny

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Summary

To test the hypothesis that enhanced immune competence of sires was associated with survival of their progeny, the immune competence of mature boars (N=87) was assessed by measuring both antibody mediated immune responses (AMIR) and cell mediated immune responses (CMIR) to commercial vaccine antigens. Based on results, boars were allocated into tertile groups for AMIR and CMIR, and a combined immune grouping (the concatenation of AMIR and CMIR groups). The association of sire immune group (IG) with independent estimated breeding values (EBVs) for direct (PREd) and maternal (PREm) pre-weaning or post-weaning (POSTd) survival of their progeny was tested. This analysis was performed using EBVs for all boars or only the subset of boars with greater than 200 progeny recorded for survival outcomes. Results demonstrated that there were significant associations between sire IG (P=0.003) or CMIR group (P=0.019) and PREd. As CMIR increased, PREd increased; this improvement was more evident when AMIR group was low. However, when only more accurately evaluated boars were included in the analysis (N=56), grouping on AMIR approached significance for PREd (P=0.104). A significant effect of sire IG for POSTd or PREm was not observed. Results demonstrated that heritable variation in some immune competence measures of sires is reflected in the survival of their progeny. Results also suggested that CMIR phenotype had a greater influence on pre-weaning progeny survival than AMIR in the animals studied; however, selection of animals with a balanced ability to mount both CMIR and AMIR remains an important goal for improving broad-based disease resistance.

Keywords: immune competence, progeny survival

Introduction

Survival of progeny through to slaughter age is a key driver directly impacting on profitability and animal welfare within the Australian Pork Industry. Over recent years, there has been an increase in the incidence of diseases in some pig populations (Eze*, et al.*, 2015), not only causing increased mortality rates, but also reduced productivity as animals recover from infection to reach pre-infection production levels (Flori*, et al.*, 2011). *Actinobacillus pleuropneumoniae* (APP) has been identified as a disease of pigs that requires repeated vaccinations to provide adequate protection against the disease, suggesting suboptimal efficacy of the vaccine and/or variable or poor immune capacity of animals receiving the vaccine. Immune competence, or the body's ability to respond to foreign antigens and render them harmless, involves a complex network of factors (Mallard*, et al.*, 1992). Since it is not possible to identify all the genes that contribute to enhanced immune competence, an alternative strategy is to consider immune competence as a quantitative trait with a measurable phenotype (Wilkie & Mallard, 1999). Procedures using test antigens (Mallard*, et*

al., 1992, Wilkie & Mallard, 1999) have been developed to assess the immune competence phenotype of livestock including pigs and dairy cattle, combining measures of an animal's ability to mount both an antibody mediated (AMIR) and cell mediated (CMIR) immune responses. Selection for enhanced overall immune competence has the potential to improve an animal's ability to cope with a variety of diseases, improve responses to commonly used vaccines, reduce health-related costs and increase productivity. SEMEX, a Canadian dairy genetic supply company (www.semex.com) commercially marketing semen from immune competence tested Dairy bulls, have shown that daughters of Immunity+™ sires (classified as above average for both AMIR and CMIR) have improved overall herd health and increased fertility.

Based on the assumption that immune competence is both variable and heritable (Mallard*, et al.*, 1998, Thompson-Crispi*, et al.*, 2013), we hypothesized that the immune competence of boars would be favourably associated with survival of their progeny reared in commercial environments.

Material and methods

Sampling of boars

Mature boars (N=87) from three different breeding lines (Maternal Line A: N=25, Maternal Line B: N= 29 and Terminal Line C: N= 33) were randomly allocated to three groups for testing immune competence. At the time of testing, these boars were current sires producing purebred progeny at a large-scale breeding company, where progeny were individually recorded for survival in both the pre- and post-weaning phases.

Assessment of immune competence

Based on previous studies (Miller*, et al.*, 2008, Harper*, et al.*, 2017) tetanus toxoid (TT) was identified as a suitable model antigen for assessment of immune competence. Prior to testing, boars had no history of vaccination with TT and a low likelihood of natural exposure to *Clostridium tetani* in the piggery environment. To assess AMIR, boars were bled on Day 0 to establish base line levels of anti-tetanus toxoid specific IgG1 serum antibody before being vaccinated with Ultravac 5-in-1 (Zoetis ®), containing the tetanus toxoid and other Clostridial antigens. Boars received a booster vaccination at Day 21 and were bled on Day 30 to measure secondary antibody responses. An in-house ELISA was developed (Miller*, et al.*, 2008), to measure antibody levels. Samples were tested across two ELISA plates (Plate 1, N= 46 boars and Plate 2, N=41 boars), where Day 0 and Day 30 samples were paired on the same plate. The phenotype for AMIR was represented as a within plate sample to positive (S/P) ratio expressed as:

$$\text{AMIR} = \frac{(\text{Sample absorbance at Day 30- Negative control mean}^{A})}{(\text{Positive control mean}^{B} - \text{Negative control mean}^{A})} \quad (1)$$

^{A} mean of the three lowest sample absorbance values on Day 0 (within plate)
^{B} mean of the three highest sample absorbance values on Day 30 (within plate)

Cell mediated immune responses (CMIR) were assessed on the same 87 boars by measuring the magnitude of a delayed type hypersensitivity (DTH) reaction to Equivac T (Zoetis ®) delivered by intradermal injection at the perineal area (Harper *et al*., 2017, in press). The magnitude of DTH reactions were assessed by measuring changes in double skin thickness at the injection site. Briefly, on Day 30 after the initial vaccination with Ultravac 5-in-1 (Zoetis ®), each boar received intradermal injections of Equivac T (Zoetis ®), containing the tetanus toxoid antigen, and saline at an adjacent injection site. At each

injection site, the double skin fold thickness (mm) was recorded in triplicate using a Harpenden Skinfold Caliper both on Day 30 (before the intradermal injection) and Day 32 (48 hours post intradermal injection), to obtain average skin thickness values. The phenotype for CMIR was defined as the difference between the double skin thickness values observed on Days 32 and 30, expressed as:

CMIR = (Antigen Day 32 thickness – Antigen day 30 thickness) - (Saline Day 32 thickness – Saline Day 30 thickness) (2)

Using these two phenotypes, boars were allocated to immune groups (IG). Three equal sized groups (N=29) for each of AMIR and CMIR were created based on the ranking of the boar for each trait, independent of the other. Allocation to an overall IG was based on the combination of these two group codes.

Estimated Breeding Values (EBVs) for pig survival

All boars had EBVs for direct (PREd) and maternal (PREm) additive genetic effects influencing pre-weaning survival, and direct effects for post-weaning survival (POSTd), expressed as a percentage. The data used to produce in-house EBVs for these traits included data recorded since 2009 on more than 500,000 piglets (historical and current), individually tagged and any incidence of mortality recorded up until day 70 after their date of birth. A bivariate linear animal model to estimate EBVs for pre- and post-weaning mortality traits accounted for birth year-quarter, breed and gender, sow parity-foster status group, and corrected broadly for groupings of piglets by birth weight, litter size and gestation length. Additional systematic effects fitted for post-weaning mortality were weaning age and weaning site. Random effects included pig (to obtain direct genetic effects for PREd and POSTd), birth and nurse litters, while the nurse sow (additive genetic and permanent environmental effects) was fitted as a random effect to obtain an estimate of PREm. For convenience, mortality EBVs have been expressed as breeding values for progeny survival in this paper.

Statistical analysis

The significance of breeding line, test cohort and assay plate, along with their two-way interactions, for AMIR (with the exception of terms for plate, CMIR) were tested using a linear model in IBM SPSS Statistics (Version 24.0). The significance of grouping for AMIR alone, CMIR alone, or overall IG for progeny survival EBVs, was tested using a model which included breeding line and IG as class effects. This analysis was performed using EBVs for all boars, or only the subset of boars with greater than 200 progeny recorded for survival outcomes. Due to the relatively small trial size in this first study (more progeny survival data will accumulate), tending towards significance was assumed for P values$\leq$ 0.10.

Results and discussion

Significant effects for AMIR and CMIR phenotypes

No significant systematic effects (line, P=0.938; test cohort, P=0.196; plate, P=0.201) were detected for AMIR, suggesting that antibody responses were not influenced by breeding line. For CMIR, test cohort had no significant effect (P=0.808) but the influence of line approached significance (P=0.103). Line C (a terminal line) had significantly higher CMIR (3.080±0.287) than the other (maternal) lines A and B (2.222±0.333 and 2.372±0.307 respectively). These results suggest that Line C may have an enhanced CMIR phenotype. No two-way interactions were significant.

Immune group allocation

Boars were equally divided into tertiles (29 per group) based on AMIR and CMIR phenotype, independently. However, boars were then allocated to the combined IG in a less balanced manner than would be expected if CMIR and AMIR were independent of each other (Table 2). The Pearson chi-square test approached significance (P=0.11), suggesting AMIR and CMIR were not independent of each other. Also, the Pearson's correlation between the AMIR and CMIR phenotype (r^2=0.333) and rank correlation between groups (r^2=0.274) were positive and significant (P=0.002, P=0.01 respectively). This result differs from findings in dairy cattle (Hine*, et al.*, 2012, Thompson-Crispi*, et al.*, 2013), where AMIR and CMIR were negatively correlated. Generally, there were more boars allocated to IG extremes (low group 11, low score for AMIR and CMIR respectively and high group 33, high score for AMIR and CMIR respectively) relative to the intermediate groups (12, 21, 22, 23 and 32); relatively fewer boars were represented in opposing extreme groups for either AMIR or CMIR (13 or 31).

The association between immune grouping and piglet survival EBVs

The significance of AMIR and CMIR grouping for estimates of PREd, POSTd and PREm is shown in Table 1. No significant differences between AMIR groups were observed when all boars were included in the analysis. However, when only more accurate boars were included (N=56), AMIR group approached significance for PREd (P=0.104). Perhaps surprisingly, there was a tendency for boars which had relatively lower antibody responses to vaccination (i.e. low score of 1) to have improved progeny survival in the pre-weaning phase. In contrast, the progeny of boars with enhanced CMIR phenotype, had consistently higher PREd, regardless of progeny numbers; with the highest responding boars having significantly better pre-weaning progeny survival than boars in the other two groups. These results suggest that at this stage of production and under the environmental conditions encountered, an enhanced CMIR versus AMIR phenotype measured using the current model had a stronger influence on improved progeny pre-weaning survival in piglets.

As CMIR competencies increased PREd increased, which was more evident when AMIR competence was low (Table 2), which supports results presented in Table 1. The comparable model containing main effects and the interaction term (AMIR×CMIR group) showed the interaction was significant (P=0.026). Our results showed that CMIR had a greater influence on pre-weaning survival of progeny than AMIR. This is probably due to several factors in the pre-weaning phase including the influence of maternal antibodies in protecting the piglet against disease (Bandrick*, et al.*, 2014), the aetiology of development of the immune system in the suckling piglet and the types of disease most commonly encountered in the piggery during this phase of the production cycle. Further investigations into the role and impact of maternal derived immunity are necessary as previous studies have demonstrated that the presence of maternal antibodies can compromise the ability of piglets to respond effectively to vaccination (Bandrick*, et al.*, 2014). With a larger sample of boars and longer duration of data recording, daughters of boars can be assessed for their maternal contributions to immune competence.

A significant effect of sire IG for post weaning survival (POSTd) or maternal pre-weaning survival (PREm) were not observed. For POSTd, IG comparisons may have been confounded by extensive post-weaning vaccination schedules, with artificially induced immune protection masking the influence of inherited variation in immune competence for health and survival. Also, mortality during this time is low. Similarly, extensive vaccination

schedules for sows pre-farrowing might also mask any association between PREm and sire IG. However, sires in the current trial also did not yet have significant numbers of daughters with litters to accurately estimate their PREm EBV. Survival assessed in a population with lowered vaccination requirements for a longer period during the finishing phase might be more informative to establish the relationships between immune competence and survival until slaughter age.

Conclusions

These preliminary analyses show that an improvement of pre-weaning progeny survival was evident for boars with higher CMIR phenotypes, but not AMIR phenotypes. This outcome supports the hypothesis that inherited variation in immune competence measures has an impact on pre-weaning progeny survival. However, there were relatively few sires with immune competence phenotypes and more robust results would be established if younger selection candidates (that are easier to handle than mature boars) could be recorded for immune competence phenotypes. More survival data on progeny of sires included in this study will assist in confirming inheritance of these measures and improve estimates of the associations between immune competence measures, production and survival traits.

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Table 1: Mean (±standard error) for pre-weaning (PREd), post-weaning (POSTd) and maternal pre-weaning (PREm) survival EBVs by group for AMIR and CMIR phenotype, for all boars or boars with greater than 200 progeny recorded for survival

		All Boars			Boars>200 Progeny		
Trait	Group	PREd	POSTd	PREm	PREd	POSTd	PREm
AMIR	1	4.2±0.5	0.7±0.3	2.1±0.3	4.9±0.6[a]	0.6±0.4	2.4±0.4
	2	3.7±0.5	0.9±0.3	1.6±0.3	3.3±0.7[b]	0.3±0.4	1.4±0.5
	3	3.3±0.5	0.8±0.3	1.7±0.3	3.2±0.6[b]	1.1±0.3	1.7±0.4
	P-Value	0.505	0.895	0.555	0.104	0.284	0.336
CMIR	1	3.3±0.5[c]	0.7±0.3	1.8±0.3	3.7±0.7[c]	0.3±0.4	2.1±0.5
	2	3.0±0.5[c]	0.9±0.3	1.5±0.3	2.6±0.6[c]	0.9±0.4	1.4±0.4
	3	4.9±0.5[d]	0.9±0.3	2.0±0.3	5.0±0.6[d]	0.8±0.4	2.1±0.4
	P-Value	0.019	0.873	0.536	0.036	0.558	0.518

[c-d] means with different superscripts within column and trait indicate significant differences (P<0.05)
[a-b] means with different superscripts within column and trait indicate significant differences (P=0.10)
superscripts are only provided when the effect is significant

Table 2: Mean (±standard error) for pre-weaning (PREd), post weaning (POSTd) and maternal pre-weaning (PREm) survival EBVs for Immune Groups for all boars or boars with greater than 200 progeny recorded survival*

Immune		All Boars				Boars>200 Progeny		
Group*	N	PREd	POSTd	PREm	N	PREd	POSTd	PREm
11	13	3.4±0.7[a]	0.4±0.4	2.2±0.5	7	4.0±0.9[c]	0.0±0.6	2.4±0.7
12	10	2.9±0.8[a]	1.3±0.5	1.5±0.6	7	3.2±1.0[c]	1.2±0.6	2.1±0.8
13	6	7.9±1.1[b]	0.5±0.6	2.7±0.8	6	7.6±1.0[d]	0.6±0.7	2.6±0.8
21	11	3.3±0.8[a]	0.9±0.5	0.9±0.6	5	3.3±1.1[c]	0.1±0.7	0.8±0.9
22	10	2.5±0.8[a]	0.7±0.5	1.4±0.6	8	2.4±0.9[c]	0.4±0.6	1.4±0.7
23	8	5.8±0.9[b]	1.2±0.6	2.7±0.7	2	7.5±1.8[d]	0.6±1.2	3.2±1.4
31	6	2.8±1.1[a]	1.0±0.6	2.6±0.7	5	3.6±1.1[c]	1.4±0.7	2.9±0.9
32	8	3.7±0.9[a]	0.7±0.5	1.5±0.6	4	2.2±1.3[c]	1.3±0.8	0.3±1.0
33	15	3.2±0.7[a]	0.9±0.4	1.5±0.5	12	3.2±0.7[c]	0.9±0.5	1.6±0.5
P-Value		0.003	0.963	0.360		0.009	0.736	0.387

* Group codes represent the concatenation of tertiles for AMIR and CMIR phenotypes respectively
[a-d] means with different superscripts within column indicate significant differences (P<0.05)
superscripts are only provided when the effect is significant