IMPROVING HEALTH OF NEONATAL PIGLETS
BY INJECTING IMMUNOGLOBULINS

Report prepared for the
Co-operative Research Centre for an Internationally
Competitive Pork Industry

By
Mr Rob Smits and Dr Sue Woon

PO Box 78
Corowa
NSW 2646

September 2011
Executive Summary

Improving the health and viability of dystrophic (light and low vitality) piglets by intramuscular injection of plasma derived from slaughtered sows of the same farm was investigated. In a series of studies, blood plasma from young parity sows at slaughter was collected and processed by a GMP facility at Bendigo, VIC, and stored at -20°C. In the pilot study, neonatal piglets (n=81) were injected with 5 mL plasma on d 1 of age with plasma collected from sows of the same farm and health status (n=490) and compared to piglets left untreated (n=83). There was no difference in weight gain between d 1 to d 14 in the two groups (average of 162 g/d; \( P = 0.49 \)), though there was a numeric reduction in mortality by d 14 in the plasma treated piglets (14 vs 17%; \( P = 0.41 \)). In a larger study, dystrophic piglets weighing between 0.8 to 1.3 kg at birth were injected with 10 mL plasma on either d 1 (n=200), or d 1 and 3 (n=209), and compared with piglets injected with a placebo (Hartmann’s solution) on d 1 and 3 (n=203). There was no significant difference in weight gain or mortality by d 7, 14 or 21. Mortality was numerically higher in piglets injected with plasma on d 1 and 3, particularly in the first week, compared to those injected with plasma on d 1 only or treated with the placebo. In the final study, the transfer of antibodies from the plasma injection method was assessed. Sows destined for slaughter were vaccinated either with a tetanus toxoid vaccine (Equivac®) or a placebo (Hartmann’s solution) two weeks prior to slaughter. The blood from each donor sow group was collected, plasma processed and stored before being injected into dystrophic piglets on d 1 and 3. The antibodies specific to tetanus were higher in piglets treated with plasma from Equivac treated sows compared to the placebo sow plasma. This demonstrated that antibodies as IgG are transferred from plasma injected into piglets. In conclusion, despite an increase in antibody transfer by the plasma injection method, there was no benefit to health or viability of neonatal piglets. Due to the absence of any demonstrable benefit of plasma therapy, and the restrictions placed on plasma harvesting in NSW and VIC, it is recommended that other strategies are adopted.
# Table of Contents

Executive Summary ......................................................................................................................... i

1. Introduction ................................................................................................................................. 1

2. Methodology ................................................................................................................................. 2

3. Outcomes .................................................................................................................................... 6

4. Application of Research .............................................................................................................. 13

5. Conclusion .................................................................................................................................. 13

6. Limitations/Risks ....................................................................................................................... 14

7. Recommendations ...................................................................................................................... 14

8. References .................................................................................................................................. 15

Appendices ..................................................................................................................................... 17

Appendix 1: ...................................................................................................................................... 17
1. Introduction

Pigs weaner per sow and subsequently sold per sow per year are key economic drivers of efficiency in pig herds. Currently, Australian producers are averaging 21.5 weaned/sow with a target of 24 weaned per sow (Pork CRC Benchmarking project). Internationally successful producers achieve between 25-27 pigs weaned per sow/year. Pre-weaning and post-weaning losses are highly variable, however we can say with confidence that on many farms that pre-weaning mortality has not been significantly reduced over time. With published values averaging between 15-20% preweaning losses of newborn piglets (Varley, 1995), the contribution of pre-weaning mortality to pigs sold/sow/year is substantial. With larger litter sizes as part of the genetic selection program, the variation of birth weight within litter may have caused a larger proportion of unviable and unthrifty piglets, possibly due to uterine crowding (Foxcroft et al., 2007).

Common causes of sucker mortality include, in order of frequency of occurrence: Overlying by sow, trauma, weakness and less than average weight, starvation and chilling and abnormalities including splay legs and blind rectum, scours of bacterial and viral origins and systemic diseases such as arthritis and pneumonia (Rooke and Bland, 2002). Gilt litters have also been identified as a substantial contribution to small piglets and possibly piglets with lower active immunity for post-weaning protection against bacterial and viral challenge (Miller et al. 2008). Unthrifty piglets usually result as a combination of birth weight, low vigour or teat competitiveness which leads to low intake of colostrum and milk. As a consequence, immunoglobulin (Ig) intake is low and also variable. Overriding the quantity and quality of colostrum and milk-derived Ig's by direct dosage of piglets has the potential to improve neonatal survival and the development of active immunity for post-weaning health. Preparations of porcine or bovine derived milk Ig's in commercial colostrum replacers when orally delivered have had limited success either due to administration through stomach tubing, a skilled technique, and the need for regular dosage (Morrison et al., Project 2B103). They also do not contain farm-specific Ig's or antibodies. Oral delivery of Ig's can also be affected by the efficiency of uptake from the digestive tract, with ‘gut closure’ occurring within 24 hours and reducing absorption across the ileum. There is a very small opportunity in the life of a neonate pig to achieve maximal immune protection from the dam. Within 24-36 hrs from birth, gut closure occurs in the newborn pig intestine which leads to a gradual decline in the absorption of maternal immunoglobulins (Rooke and Bland, 2002). Gut closure inhibits the transfer of macromolecules like immunoglobulins to be absorbed into the circulation (Rooke and Bland, 2002). It is a defense mechanism to inhibit any passage of foreign molecules into the system.

The aim of plasma therapy is to give the neonate an added boost in its short term immune response. Plasma therapy refers to the administration of plasma which is collected from immunized animals and given directly to the piglets (Berghman et al., 2005). By injecting plasma into the animal, the limitation of a short window for uptake
of plasma antibodies by oral route due to gut closure is removed. The main immunoglobulin component in colostrum is IgG, which makes up for 80% of the Igs as compared to IgA and IgM (Straw et al., 2006). The majority of IgG in colostrum is serum-derived (Bourne and Curtis, 1973), so through plasma therapy, it is possible to provide added protection to the neonates, especially piglets which have taken longer from birth to first suckle and will not have obtained as much colostrum as their other litter mates. Plasma therapy was a popular practice prior to the widespread use of antibiotics since the 1930’s (Casadevall, 1996). In a study conducted by Normantiene et al (2000), serum was collected from sows and was injected intramuscularly to the neonatal piglets at the same farm. As a result, their results showed that the usage of serum therapy halved the mortality rate of the dystrophic piglets.

Although the concept of farm-specific serum therapy has been around for decades, there is virtually nothing published. In pilot trials at APFG and Rivalea, sow blood from old, non-infectious sows has been collected and serum produced. Neonatal piglets and weaners at both sites have benefited from therapeutic treatment with site-specific serum and shown very impressive recoveries. There are many production considerations that remain to be tested under field conditions and quantification of why the pigs respond in such a way is needed. The objective of this project was to determine the Ig characteristics of plasma collected from different ages of pigs and then commercially evaluate response of neonates to plasma therapy in terms of mortality and pre-weaning growth performance.

2. Methodology

In order to allow the research program to commence a trial permit from Australian Pesticides and Veterinary Medicines Authority (APVMA) was required (see appendix). The approval for this took a considerable amount of time, and consequently delayed the project. The treatment of the harvested plasma imposed as part of the permit compliance was also more involved than was originally intended and also compared to the study methodology described by Normantiene et al (2000). The conditions of the permit stated that the plasma had to be collected from the farm where it was to be administered and had to be processed through a series of microfilters in a NATA accredited laboratory.

Measurement of IgG, IgA and IgM
A series of experiments have been done to optimise the dilution factors of sample and HRP-antibodies for IgG, IgA and IgM. Porcine IgG, IgA and IgM ELISA quantitation sets (Bethyl Laboratories Inc, USA) were used. Using the ELISA for IgA and IgM, most of the readings of the samples cannot be interpolated as the values fall beyond the standard curve. Many attempts have been made including using the recommendations from previous publications and those given by a lab assistant in Murdoch University which had experience using the Bethyl kits, the ELISA results were not favourable. Hence, due to time constraint and several unsuccessful trials at obtaining the optimal dilution factor, the decision was made to leave out the ELISA testing of IgA and IgM in the study and concentrate on quantifying IgG.

Study 1 (09V040C):  Evaluation of immunoglobulin levels in different ages of slaughter pigs.

The first part of the project was to identify the best source of plasma to source for high level of immune-protection. Blood was collected by venapuncture of the jugular vein on restrained female pigs (Framstad et al., 2000) as an 8 mL sample and tested for IgG, IgA, and IgM immunoglobulins. The age groups evaluated were:

- 10 week old growers
- 20 week old finishers
- Gilts 6-9 weeks gestation
- Parity 1 6-9 weeks gestation
- Parity 5 6-9 weeks gestation

Blood samples were collected in EDTA vacuutainers on ice packs and held at 5°C then centrifuged at 2000 g for 5 mins, sera poured off and stored at -80°C.

A sandwich ELISA was used to detect and measure immunoglobulins in serum or plasma samples. Porcine IgG, IgA and IgM ELISA quantification sets (Bethyl Laboratories Inc, USA) were used.

Study 2 (09V039C):  Pilot study to determine response to plasma treatment

The second study was established as a pilot study to inject a small number of piglets with plasma collected from sows of the same farm. The plasma was harvested from culled sows and treated as for Study 1. The average parity of the sows harvested was 1.4. The piglets included in the study were selected from gilt and runt litters. Piglets were selected within 24 h of birth and allocated within litter to either a control (non treated) or treated with 5 mL of plasma at d 1 and d 3 of age as an intramuscular
injection into the neck. Piglets were individually weighed at d 7 and d 14. Deaths within the period between treatment at d 1 and d 14 where recorded.

Study 3 (10V019C): *Improving piglet health through plasma therapy*

In a study at the Research Unit, Corowa, 612 piglets in the weight range of 0.8 to 1.3 kg birth weight were assigned to one of three experimental treatments: Control injected with 10 mL Hartmann’s solution (placebo) on day 1 and 3 of age; filtered plasma injected i.m. 10 mL given on day 1 and 10 mL of Hartmann’s solution given on day 3 (Plasma d 1); filtered plasma injected i.m. as 10 mL on day 1 and day 3 (Plasma d 1 and 3). Piglets within a litter were allocated based on their birth weight falling within 0.8 to 1.3 kg range and randomized between treatments. Treatments were represented within litter, and to avoid bias between treatments sucking their birth dam, all piglets on treatment were fostered with 24 h of birth as part of the experimental design. Piglets were injected at 4 points as 2.5 mL to minimize site injection reaction. Additional piglets that were not part of the assessment were left on the sow to make up litter size to 10 - 12. A subset of 160 piglets were serially bled at d 1, d 3 prior to plasma and Hartmann’s administration, and again on d 7. Individual weight gain and mortality were recorded at d 7, d 14 and d 21.

Study 4 (10V059C): *Tetanus toxoid antibody study*

This final study was an assessment of the transfer of antibodies from sow-derived plasma to piglets. Using the antibodies for tetanus toxoid vaccine, Equivac® (Pfizer Animal Health) in treated sows, the level of antibodies absorbed by the piglets was quantified. Equivac® is a registered vaccine for use in pigs. The birth dams had no previous exposure to Equivac®. Twelve culled sows (n=6 per treatment) were either vaccinated with Equivac® or Hartmann’s solution (placebo) on d 1 and d 14. On d 28 sows were slaughtered at a commercial abattoir and blood collected per sow. The filtered plasma was pooled within treatment and stored at -20°C. Plasma from either Control sows or vaccinated sows was then used as the treatment in plasma treated piglets. At birth four piglets weighing between 0.8 to 1.3 kg at birth were fostered onto another sow within 24 h. Of these four fostered piglets, two were selected as Control and injected with plasma either collected from Control sows, and two fostered piglets treated with plasma from Equivac® vaccinated sows (Vaccinated). In total five sows were used in the study with fostered piglets, such that there were 10 piglets treated with Control sow plasma, and 10 piglets treated with Vaccinated sow plasma. 10 mL was injected intramuscularly on d 1 and d 3. Serum samples were collected from the piglets before treatment on d 1, d 3 and again at d 7, d 14 and d 21 and tested for the specific antibodies for tetanus.
Blood was collected from donor sows (parity 1-3) within the same experimental farm at an abattoir in Laverton, Victoria. Donor sows are individually identified at the abattoir by their sow tags found on the ears. Only healthy sows with normal rectal temperatures of 38.8°C ± 0.30°C and inspected by AQIS officers at the abattoir will be used as donor sows.

The blood was collected in cleaned and disinfected 20 L containers with Terminator (Active ingredients: Glutaldehyde 15% and Cocobenzylmethylyammonium chloride (QAC) 10%) at a ratio of 1:150. Buckets are then rinsed twice with water and then dried. The container is lined with an anticoagulant, EDTA, 10g of EDTA is dissolved in 120ml of distilled water.

Approximately 10 L of blood from each sow was collected in the EDTA lined container. The containers were then sealed and transported to an APVMA approved laboratory for plasma processing (ACE Laboratories, Bendigo, VIC). At the laboratory, the blood was left overnight in the fridge at 4°C to allow cells to settle. The liquid portion of the blood was then decanted and spun in refrigerated centrifuge at 2500rpm for 30 mins. It was then poured through a sieve before centrifuging again for a further 10 mins at the same speed. The plasma was then filtered by membrane filtration. The plasma was then stored in sterile non-pyrogenic bags at -20 degrees until further use.
3. Outcomes

The level of IgG in plasma collected from a range of pig ages was relatively consistent (Table 1). Parity 1 sows were numerically the highest source of plasma IgG, and they also were the least variable between sows. Therefore, it was decided that all blood samples would be from young parity sows. In terms of availability, a high turnover of sows after returns and failing pregnancy testing are culled provided enough plasma for subsequent testing, and is a potential source of plasma for commercial use.

The pilot study conducted at a commercial unit at Corowa on growth and mortality of a small sample of litters (15 litters in total) is summarized in Table 2. Weight gain and mortality was unaffected by plasma treatment. The average birth weight was within expectations for gilts (Miller, 2006), but were not of the lowest birth weights at risk of pre-weaning mortality identified by Morrison et al (unpublished, Pork CRC project 2B-101) as less than 1.3 kg birth weight.

The growth and immunity responses to injecting plasma at either day 1 and/or day 3 are presented in Table 3 and 4. As in the pilot study, there was no discernable improvement in growth rate or mortality in piglets treated with plasma. Although piglet weight at 21 d was 5% heavier, growth rate over the period was unaffected by treatment (Table 3). Piglet losses were higher in the group treated with plasma at d 1&3 (Table 4), and the lighter piglets in this treatment were more likely to die before reaching their 21 d weight assessment. Immune IgG levels did not respond to injected plasma by day 3 (prior to the d 3 injection) nor was there any difference in piglet IgG by day 7 (Table 4). The mortality level in the first 14 days, particularly the first 7 d, was considerably higher in the treatment injected with plasma on both d 1 and 3. There was a similar mortality by 14 d and 21 d between controls and piglets injected with plasma on d 1 only. All piglets were picked up and handled the same with the Controls injected with the placebo on d 1 and 3. Although the level of mortality was higher in this study than the pilot study (Table 2), the weight of piglets allocated to the present study was much lighter and deemed to be at risk.

The final study was designed to quantify the level of transfer of antibodies from sow plasma to neonatal piglets. The antibodies to tetanus toxoid, administered as Equiviac®, were significantly increased in piglets injected with sow plasma from Equivac® vaccinated sows (Figure 1). This confirms that antibodies from sows are transferred to piglets treated with plasma. Antibodies remained significantly elevated well beyond the last injection of plasma on d 3, however the peak in concentration occurred on d 3, prior to the second injection of sow plasma. By d 21, antibody levels began to return to the levels of piglets treated with plasma from unvaccinated sows.
The laboratory also assessed the gross changes in plasma characteristics in terms of protein, albumin and gross globulin before and after filtration (Table 5). The results indicate that there was a loss of approximately 30% of protein and antibodies as a result of the filtration process.

Table 1. Quantification (mean ± SD) of immunoglobulin levels (IgG) from a sample (n=5) of grower-finishers, gilts and sows (Study 1)

<table>
<thead>
<tr>
<th>Age group</th>
<th>IgG</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grower - 10 week old</td>
<td>2.11 ± 0.21</td>
<td>2</td>
</tr>
<tr>
<td>Finisher 20 week old</td>
<td>2.06 ± 0.25</td>
<td>4</td>
</tr>
<tr>
<td>Gilt</td>
<td>2.07 ± 0.20</td>
<td>3</td>
</tr>
<tr>
<td>Parity 1</td>
<td>2.30 ± 0.14</td>
<td>1</td>
</tr>
<tr>
<td>Parity 5</td>
<td>2.02 ± 0.34</td>
<td>5</td>
</tr>
</tbody>
</table>


Table 2. Daily weight gain and mortality of neonatal piglets treated with 5 mL plasma on d 1 and d 3 intramuscularly (Plasma) compared to untreated controls (Control) (Study 2)

<table>
<thead>
<tr>
<th></th>
<th>Untreated Control</th>
<th>Plasma treatment</th>
<th>SED</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number allocated, piglets</td>
<td>83</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight day 0, kg</td>
<td>1.43</td>
<td>1.41</td>
<td>0.03</td>
<td>0.50</td>
</tr>
<tr>
<td>Weight day 7, kg</td>
<td>2.52</td>
<td>2.55</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Weight day 14, kg</td>
<td>3.80</td>
<td>3.65</td>
<td>0.16</td>
<td>0.34</td>
</tr>
<tr>
<td>ROG day 0-7, g/day</td>
<td>156</td>
<td>163</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>ROG day 0-14, g/day</td>
<td>165</td>
<td>158</td>
<td>0.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Mortality 0-14 d, % of allocated piglets</td>
<td>16.8</td>
<td>14.1</td>
<td>$\chi^2$ 0.67, 0.41</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Daily weight gain of light weight neonatal piglets treated with either 10 mL plasma or placebo on d 1 and d 3 intramuscularly (Study 3)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Plasma day 1</th>
<th>Plasma day 1 and 3</th>
<th>SED</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. allocated, piglets</td>
<td>203</td>
<td>200</td>
<td>209</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piglet live weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>1.13</td>
<td>1.14</td>
<td>1.12</td>
<td>0.01</td>
<td>0.186</td>
</tr>
<tr>
<td>day 7</td>
<td>1.85</td>
<td>1.85</td>
<td>1.84</td>
<td>0.02</td>
<td>0.969</td>
</tr>
<tr>
<td>day 14</td>
<td>2.95</td>
<td>2.99</td>
<td>2.99</td>
<td>0.04</td>
<td>0.901</td>
</tr>
<tr>
<td>day 21</td>
<td>4.52</td>
<td>4.57</td>
<td>4.75</td>
<td>0.06</td>
<td>0.270</td>
</tr>
<tr>
<td>ROG day 1-14, g/day</td>
<td>129</td>
<td>131</td>
<td>132</td>
<td>2.90</td>
<td>0.919</td>
</tr>
<tr>
<td>ROG day 1-21, g/day</td>
<td>156</td>
<td>161</td>
<td>171</td>
<td>2.85</td>
<td>0.172</td>
</tr>
</tbody>
</table>
Table 4. Immunoglobulin status as IgG in subset (n=46 per treatment) prior to treatment at d 1, 3 and mortality by d 3,14 and 21 of light weight neonatal piglets treated with either 10 mL plasma or placebo on d 1 and d 3 intramuscularly (Study 3).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Plasma day 1</th>
<th>Plasma day 1 and 3</th>
<th>SED</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG day 1, mg/mL</td>
<td>22.0</td>
<td>25.3</td>
<td>23.8</td>
<td>1.06</td>
<td>0.448</td>
</tr>
<tr>
<td>IgG day 3, mg/mL</td>
<td>17.9</td>
<td>22.5</td>
<td>19.5</td>
<td>1.02</td>
<td>0.190</td>
</tr>
<tr>
<td>IgG day 7, mg/mL</td>
<td>18.8</td>
<td>18.8</td>
<td>16.4</td>
<td>0.95</td>
<td>0.498</td>
</tr>
</tbody>
</table>

Mortality

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number at d 1</td>
<td>203</td>
<td>200</td>
<td>209</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1-7, % at d 1</td>
<td>11.8</td>
<td>14.0</td>
<td>18.7</td>
<td>χ² 3.98</td>
<td>0.137</td>
</tr>
<tr>
<td>Day 7-14, % at d 7</td>
<td>11.7</td>
<td>12.8</td>
<td>12.9</td>
<td>χ² 0.14</td>
<td>0.932</td>
</tr>
<tr>
<td>Day 14-21, % at d 14</td>
<td>10.1</td>
<td>6.7</td>
<td>8.1</td>
<td>χ² 1.22</td>
<td>0.543</td>
</tr>
<tr>
<td>Day 1-14, % at d 1</td>
<td>22.2</td>
<td>25.0</td>
<td>29.2</td>
<td>χ² 2.71</td>
<td>0.258</td>
</tr>
<tr>
<td>Day 1-weaning, % at d 1</td>
<td>30.5</td>
<td>30.0</td>
<td>35.9</td>
<td>χ² 2.00</td>
<td>0.368</td>
</tr>
</tbody>
</table>

Table 5. Effect of filtration process on plasma characteristics for total protein, albumin and gross globulin count of plasma collected from sows vaccinated with Hartmann’s solution (Control) or Equivac® (raw data).

<table>
<thead>
<tr>
<th>Filtration stage at sampling</th>
<th>Control</th>
<th>Equivac</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein g/L</td>
<td>77</td>
<td>59</td>
<td>74.5</td>
</tr>
<tr>
<td>Albumin g/L</td>
<td>42</td>
<td>34</td>
<td>40.5</td>
</tr>
<tr>
<td>Gross globulin g/L</td>
<td>35</td>
<td>25</td>
<td>34.0</td>
</tr>
</tbody>
</table>

% loss due to filtration 28 26 31
Figure 1. Antibody response to tetanus toxoid in plasma of piglets treated with sow plasma from Control sows (placebo) or Vaccinated sows immunized for tetanus (Part 4)
Discussion

The treatment of piglets with injected sow plasma at d 1 and 3 of age was successful in increasing circulating antibodies in treated piglets derived from donor sows. The use of the tetanus toxoid vaccine to the donor sows and testing for specific antibodies to the vaccine confirmed that prior to either Control or Equivac® plasma treatment on d 1 from donor sows, there was a similar level of baseline immunity to tetanus in the neonate. Over time, the level of immunity steadily declined in the piglets treated with plasma from Control sows whereas the plasma from the vaccinated sows increased following injection on d 1. Interestingly, there was no discernible increase in piglets treated with vaccinated plasma measured at d 7 following plasma treatment on d 3. The results from Study 3 also showed there was no improvement in performance or health of piglets treated with plasma on d 3 as well as d 1. There may be changes to the neonatal immune system that suppresses the response to plasma treatment after day 1. The level of IgG transfer declines after 24 h due to a reduced absorption of colostrum-derived IgG from the small intestine (Rooke and Bland, 2002). Although the piglet is born with the necessary components to produce its own immunoglobulins, these components are functionally underdeveloped (Butler et al., 1981). The lack of response to further treatment with sow plasma in Study 4 may have been due to a suppression of active synthesis by the piglet, as it has been reported that absorption of maternal IgG from the gut may repress active synthesis until after gut closure (Rooke and Bland, 2002). Though insignificant, there was a numerical reduction in IgG levels in the piglets treated with plasma on both d 1 and 3 in Study 3. A possible explanation is that treating piglets after a certain age with sow plasma may reduce IgG antibodies due to a reduced endogenous IgG synthesis within the piglet.

The results of Study 3 did not support the hypothesis that piglets treated with sow plasma would perform better than control piglets. The piglets used in Study 3 were selected as being of low birth weight and hence high risk of mortality. There was no benefit in terms of weight gain or survivability in the plasma treated piglets compared to piglets treated with a placebo as Hartmann’s solution. There was also no effect on circulating IgG following plasma treatment in the treatment group Plasma d 1 compared to piglets given the placebo on d 1. This result does not support the hypothesis that injected plasma therapy with the levels injected increases circulating IgG. There was some indication from the pilot study (Study 2) on another unit that treated piglets were less likely to die (14 vs 17% by 14 d). This effect was not repeated in the larger Study 3. There were some differences in the experimental methodology between the two studies. Firstly, the dosage of plasma was 5 mL on d 1 and 3 in Study 2 whilst it was increased to 10 mL in Study 3, and given at either d 1 or d 1 and d 3. Secondly, there were some piglets that were treated which were unfostered, whereas in Study 3 all piglets on trial were fostered. This was done to remove any non-treatment effects between individual piglets that were fostered or unfostered, which were unrecorded in Study 2. The level of pre-weaning mortality from the farm records where the pilot
study was conducted was lower overall than the average farm pre-weaning mortality where Study 3 was conducted, indicating a possible difference in hygiene and husbandry. Finally, the weight selection of the experimental piglets in Study 3 were smaller and of a lower vitality than the average used in Study 2. Again this was part of the experimental design to assess the effect of plasma treatment on dystrophic piglets.

The impact of pathogen load in the farrowing environment is also likely to influence the outcome of the immune response when piglets are treated with sow plasma. Both farm environments were of low hygiene status when the experiments were conducted, and the level of mortality due a range of causes reflects this. It is probable that when the piglets, either small and dystrophic or of normal size at birth, are subject to a high pathogen load from their environment, the challenge to their immune system may be too large to benefit from antibody transfer from injected plasma.

There was some question over the effect of the processing and preparation of the plasma product as part of the APVMA permit, such that the extra filtration might have reduced the level of immunoglobulins in the plasma product used in Study 3. The results from Study 4 showed that specific antibodies to tetanus were still able to be transferred via the filtered plasma. We did not test the level of IgG between filtered and unfiltered plasma, which would have provided a clearer indication if the filtration process per se resulted in a reduction in antibodies. However, the indications from the laboratory tests on protein and gross globulin levels before and after filtration suggest that the filtration process reduced antibody levels by 30%. The level of plasma to treat dystrophic piglets is also unresolved. Injecting 10 mL per piglet at weights less than 1.3 kg was deemed the maximum dose, and at this level the dose it was necessary to spread the dose over four injection sites to address welfare concerns from the Animal Ethics Committee. Due to the APVMA permit conditions, large quantities of sow blood were unable to be collected and pooled according to IgG status as originally proposed. It was therefore not possible to investigate dose responses between high IgG and low IgG plasma samples. Increasing the dose of plasma would require a more concentrated form of plasma, such as re-constituted freeze dried plasma, which fell outside of the permit for this trial project.

The measurement of IgA and IgM immunoglobulin was problematic. These Ig’s have been found to be difficult to assay in the past (Miller, pers. comm.). The routine measurement of IgG was achievable at Charles Sturt University. However, the assays consumed a lot of time from Dr Woon, the Masters student involved in the project, and would be unlikely to be a cheap assessment on a routine basis.

As the direct injection of sow plasma was observed to be largely unsuccessful in improving piglet health and viability, other strategies need to be considered. If an oral form of spray dried plasma was able to be delivered to the neonatal pig, this could be a possible means of transferring specific on-farm antibodies to piglets in the form of a colostrum replacer. Improving piglet birth weight either by reducing the proportion of
gilts in the herd that farrow or using nutrition or metabolic modification are other options. Smits (2011) identified that gilt litters contribute towards a poorer HFC due to low birth weight litters on average compared to older sows. Meanwhile, Gatford et al. (2010) was able to increase piglet birth weight in gilts and sows by the use of a daily injection of pST (porcine somatotropin) from d 25. As piglet birth weight has been identified as the main factor associated with poor weight gain throughout life and risk of mortality by Morrison et al (Pork CRC project 2B-103), strategies that reduce low birth weight pigs will likely reduce neonatal mortality. However, there will always be some proportion of piglets that are born lighter than ideal and consequently at risk of overlay or poor viability due to insufficient colostrum intake. Improving the hygiene level of the environment where neonatal piglet is reared is another strategy to minimize the disease risk to these piglets at risk. Attention to husbandry detail; minimal fostering; and high hygiene levels pre-farrowing, during farrowing and within the first week of life are critical elements to improve piglet health. In summary, efforts made to reduce the potential pathogen load; increase birth weight; and increase colostrum intake are management and husbandry strategies that are recommended. Further improvements to health and viability under these conditions by injecting plasma from on-farm blood sources remains to be evaluated. However, the risk of further intervention in picking up piglets and injecting them in their first days of life could be disruptive and risk environmental contamination, negating possible benefits from the plasma derived antibodies.

This project formed the basis for a Master of Science degree by Dr Siewyin Woon through Charles Sturt University (Supervised by Dr Thiru Vanniasinkam and Prof Mary Barton of University of SA)

4. Application of Research

The project was unsuccessful in delivering an improved growth performance and reduction in mortality in piglets injected with filtered plasma collected from sows of the same farm. The project was able to demonstrate that filtered plasma products can increase antibody transfer to neonatal piglets injected with 10 mL on day 1 and 3 of age. However this had no effect on vitality or health of piglets deemed to be at high risk of mortality.

5. Conclusion

The project concluded that the use of sow plasma as a i.m. injection within 72 h of life to increase the health of neonatal piglets was unsuccessful. The transfer of antibodies by the direct injection method was able to be demonstrated, however it is proposed
that either the environmental pathogen load was too high, or the level of immunoglobulins too low, to be an effective alternative treatment to reduce mortality and improve weight gain under the farm conditions evaluated.

6. Limitations/Risks

The studies were conducted under commercial conditions prevailing in the farms at the time, and since have been identified as having low hygiene and high environmental pathogen levels. The response to higher doses of on-farm antibodies derived from plasma identified as having high levels of IgG was untested. The restricted use of farm derived sow plasma that needed to be produced in a certified GMP (Good Manufacturing Practice) laboratory under APVMA permit limits the application of further evaluations and on-farm demonstration.

7. Recommendations

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

1. There was no supporting evidence from these studies that the health or weight gain of piglets pre-weaning is improved by injecting plasma products derived from the slaughtered sows located on the same farm.

2. Further studies using farm-derived porcine plasma products in NSW and VIC will be limited by the need for APVMA permit for use of the products. Given the data from the current project, there is a lack of supportive evidence to renew the application for another trial permit application for use in these states. Therefore it is recommended that if further evaluations are proposed, the project is studied elsewhere.

3. Other strategies such as proper hygiene, husbandry practices that promote colostrum intake and improving piglet birth weight and vitality should be explored as a priority.
8. References


Blackwell Publishing Ltd.

Appendix 1 - Notes

Confidential Information

If a Final Report contains Confidential Information:

- the Researcher must indicate on the cover of the final Report that the Final Report contains Confidential Information
- the Pork CRC may request the Researcher to produce a non-confidential version of the Final Report in a form suitable for general distribution, and the Researcher must do so within 28 days of receiving the request

Deficient Report

If the Pork CRC reasonably forms the view that the Final Report does not adequately set out matters referred to, it must notify the Researcher of the extent to which it believes the Final Report is deficient.

Ownership of Reports

The Researcher will own copyright in all Reports, but not the Project Outcomes described in the Reports. The Researcher grants to the Corporation a perpetual, irrevocable, fully paid, royalty-free, worldwide licence to use the Reports and the information disclosed in them and any other copyright material provided with the Reports for the Corporation’s purposes, including reporting to its stakeholders, including the government.
Appendices

*Appendix 1:*

Appendix  APVMA permit