AN ASSESSMENT OF AN ATTENUATED LIVE STREPTOMYCIN-DEPENDENT *ACTINOBACILLUS PLEUROPNEUMONIAE* (APP) VACCINE (SEROVAR 15) DELIVERED EITHER INTRANASAL OR AS A COMBINATION OF INTRANASAL AND INTRAMUSCULAR INJECTION (PROJECT 8C-013)

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(APRIL)

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Executive Summary

Respiratory disease due to *Actinobacillus pleuropneumoniae* (APP) is a major problem for the Australian pig industry. The current control strategies are based on multiple vaccinations combined in some cases with antimicrobial treatments. The University of Queensland have developed a modified (attenuated) streptomycin-dependent live APP vaccine, which should reduce the need for multiple vaccinations and remove the need for supportive antimicrobial treatments.

The aim of this experiment was to test the efficacy of this attenuated strain in a controlled Challenge Study in a commercial facility in a herd with endemic APP. Pigs were allocated to one of four groups of controls and vaccinates. After the vaccination, these animals were intentionally challenged with the virulent parent strain of this vaccine to induce the potential onset of disease. Extensive monitoring of these animals detailing the diseases progression in each group determined the efficacy of the vaccine.

Treatments:

TMT A. Vaccinated (2mL attenuated APP; streptomycin dependent live APP15 vaccine containing 10^8CFU/mL) via 2 ml intranasal (IN) injection 3 days prior to weaning.

TMT B. Vaccinated (2 mL attenuated APP; streptomycin dependent live APP15 vaccine containing 10^8CFU/mL) via intranasal (IN) injection 3 days prior to weaning + 2 mL intramuscular (IM) at 28 days later.

TMT C. Commercial vaccinated (ACE Laboratories, Bendigo 1mL killed APP15 autogenous)- current commercial vaccination schedule (9, 12 and 15 weeks of age).

TMT D. Negative control. No vaccination

The current commercial APP15 vaccine provided superior immune protection compared to the new attenuated live vaccine. Challenging non-naïve grower pigs with live APP of 2mL x10^{10} intranasally invoked a mild exhibition of clinical symptoms. Pigs treated with either the streptomycin-attenuated live APP vaccine administered IN (Tmt A) or a combination of attenuated live APP vaccination delivered IN+IM (Tmt B) did not prevent any clinical signs or reduce the occurrence of intervention medication. Further interpretation of the results is limited due to the low numbers used in this challenge study.

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1. Introduction

Respiratory disease due to *Actinobacillus pleuropneumoniae* (APP) is a major problem for the Australian pig industry. The current control strategies are based on multiple vaccinations combined in some cases with antimicrobial treatments – meaning that these strategies are both expensive and difficult to implement. To address these issues, the University of Queensland have developed a modified (attenuated) streptomycin-dependent live APP vaccine, which should reduce the need for multiple vaccinations and remove the need for supportive antimicrobial treatments. The new candidate vaccine strain has been tested for safety and has been proven to be safe (Turni, 2016; personal communication). Experiment number 16V065C 'Efficacy of attenuated APP vaccine (Serovar 15) in naive pigs' was conducted to validate this vaccine for naïve pigs and was a proof of concept trial. Its results cannot be transferred directly to an APP positive herd as these pigs will have maternal derived antibodies (MDA) of unknown levels. MDA levels have the potential to interfere with immune protection derived from vaccines.

In order to test vaccine efficacy, this study used pigs from a commercial farm that has an *A. pleuropneumoniae* problem and as such, the pigs will have maternal immunity to *A. pleuropneumoniae*. Pigs were allocated to one of four groups of controls and vaccinates. After the vaccination, these animals were intentionally challenged with the virulent parent strain of this vaccine to induce the potential onset of disease. Extensive monitoring of these animals detailing the diseases progression in each group will determine the efficacy of the vaccine.

Objectives:
-Evaluation of the efficacy of an attenuated live APP vaccine to control APP in a commercial herd
-Preliminary assessment of two different commercial vaccination protocols (IN pre-weaning vs IN pre-weaning + IM 4 weeks post-weaning) on attenuated live APP vaccine
-Identification and demonstration of diagnostic measures for surveillance of an effective attenuated live APP vaccination program
-Confirmation that a challenge dose of APP at 1 x 10^8 is sufficiently high enough to elucidate a clinical response in pigs seropositive to APP15

The aim of this experiment was to test the efficacy of this attenuated streptomycin-dependent live APP vaccine in a controlled Challenge Study in a commercial facility in a herd with endemic APP.

2. Methodology

All animal procedures were conducted with prior institutional ethical approval under the requirement of the NSW Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organisation/Australian Animal Commission 'Australian code of practice for the care and use of animals for scientific purposes.

APVMA 80672 Permit was obtained for 50 pigs to enter the food chain.

The experiment was conducted from March-August 2018 at Rivalea Australia, Corowa. Originally the experiment planned to use 90 pigs in total over two time replicates. However, after the first replicate of 50 pigs a decision was made not to proceed further due to the new vaccine being ineffective.

Fifty pigs were selected from the 'PigSAFE' (loose) farrowing shed which allowed determination of dam APP serology status for each trial pig. Sourcing pigs from these loose pens meant that no nose to nose contact between neighbouring litters occurred during the lactation period (solid walls between pens). All pigs used in this trial were born within 7 days and each TMT group was allocated equal body weight ranges.

The 50 mixed sex, Large White x Landrace pigs from a total of 13 dams (5 gilts & 8 sows parities 2-5) were selected. Antibody serum levels for APP15 of the five gilt dams ranged from S/P% of 82.4 to 115 whereas APP15 Antibody S/P% values in the sows ranged from S/P% of 72.4- 148.8. The four treatment groups therefore contained pigs from dams with very similar parity structure as it is recognised that gilt progeny receive reduced amounts of IgG in colostrum.

The pigs were weaned into an isolated shed that contained 4 pens (5m x 3.3m). Treatment A-C were stocked at 15 pigs/pen ($1.1m^2$/pig) and Treatment D had 5 pigs/pen ($3.3m^2$/pig) (see Diagram 1).

All commercial weaner, grower/finisher diets were fed *ad lib* during the experiment and contained Salinomycin 60ppm only. At no time during the trial did pigs receive antibiotics in water or injectable blanket treatments. Emphasis on good air quality with reasonable thermal comfort and access to clean water and feed was ensured.

Treatments:

TMT A. Vaccinated (2ml attenuated APP; streptomycin dependent live APP15 vaccine containing 10^8CFU/mL) via 2 mL intranasal (IN) injection 3 days prior to weaning.

TMT B. Vaccinated (2 ml attenuated APP; streptomycin dependent live APP15 vaccine containing 10^8CFU/mL) via intranasal (IN) injection 3 days prior to weaning + 2 ml intramuscular (IM) at 28 days later.

TMT C. Commercial vaccinated (ACE Laboratories, Bendigo 1mL killed APP15 autogenous)- current Corowa vaccination schedule (9, 12 and 15 weeks of age).

TMT D. Negative control. No vaccination

Blood samples were collected via jugular venipuncture at 3 days before weaning, 5 days after weaning (corresponds to 7d after vaccination), 63 days of age, 98 days of age, 105 of age, 112 d of age and market. Blood samples were analysed by ELISA for (APX IV toxin) as a disease response indicator.

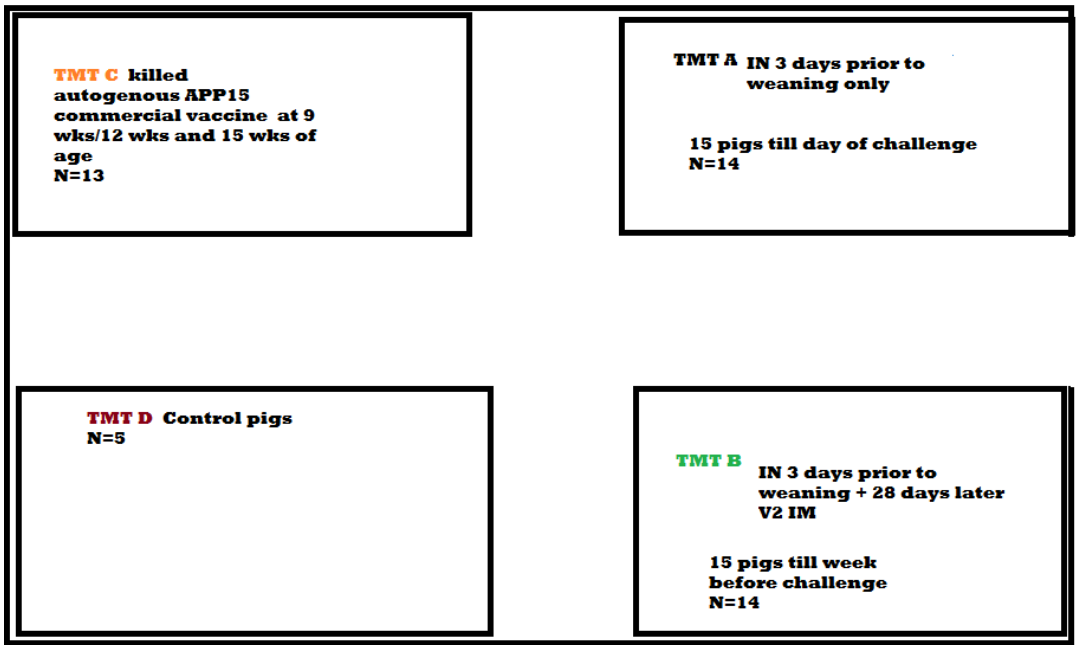

Diagram 1 : Treatment group allocation into the four pens

Disease Challenge:

At 14 weeks of age (98 days old) all treatment groups received a challenge dose of 2 mL x10^{10} cfu/mL of APP15 vaccine parent strain via route of an intra-nasal aerosol.

An intensive, 24-hour monitoring period of 7 days followed the challenge administration during this time a total of 30 observations were recorded. A single observation included a check for clinical symptoms of all pigs and a scoring/action system was designed to ensure human endpoints:

Interpretation of scoring		
Score	**Description**	**Action**
0	Normal	No action
1	Moderate changes	Animal put on alert list
1 - in multiple categories	Moderate changes	If more than two clinical signs confirmed at score 1 a broad spectrum antibiotic and a NSAID are administered
2 - in any category	Significant changes	Increase monitoring to 4 hourly
2 - in multiple categories	Significant changes	Inform project leader
3 - in any category market with ***	Severe symptoms	**Euthanise/Humane endpoint**
***	A **score of 2** in categories *nasal discharge, coughing, anorexia, laboured breathing, lethargy and reddened conjunctiva cyanosis*	**Administer a broad spectrum antibiotic and a NSAID**
	A **score of 3** in categories *nasal discharge, coughing, anorexia, laboured breathing, lethargy and reddened conjunctiva cyanosis*	**Euthanise/Humane endpoint**
Total score of 10-14	Multiple escalating symptoms	**Euthanise/Humane endpoint**

Table 1: Action points for clinical scores during the intensive monitoring period

Lung scores were obtained after collection of plugs (lungs, hearts) and scored without knowledge of treatment groups by the scorers for presence or absence of pericarditis and categorised into a lung score category as per Table 2 below.

0	healthy lung (no gross lesions noted)
1	< 10 % lung involved with localized congestion only
2	10% - 25% lung involved with consolidation or congestion only and no necrosis or pleurisy lesion
3	25% to 40% involved with some consolidation or congestion, mild pleurisy lesions and congestion
4	40 - 50% lung involved with some consolidation or congestion, mild pleurisy lesions and congestion
5	50% to 75% lung involved with some consolidation or congestion, pleurisy lesions and congestion
6	75% to 100% lung involvement with pleuropneumonia and consolidation, extensive pleural adhesions and necrotic lesions

Table 2: APP lung lesion scoring at slaughter

3. Outcomes

The pigs in TMT B showed an adverse reaction to the administration of the live attenuated vaccine 2mL x 10^8 given IM at 8-9 weeks of age which was reported to the Rivalea Animal Ethics committee.

TMT Group C encountered two commercial losses early in the trial period while groups A & B remained in groups of 15 pigs up to the day of challenge when each of these groups lost one pig (leg injury and a hernia) therefore entering the challenge period with 14 pigs for groups A & B.

TMT Group D (negative control): This group of five pigs was housed in the same size pen than TMT groups A, B and C. This group therefore had a reduced stocking density when compared to the other three treatment groups which may explain the relative mild clinical response to the challenge.

None of the experimental pigs died or reached a clinical score that necessitated euthanasia as a consequence of the challenge with an APP15 virulent dose of 2mL x 10^{10}cfu/mL administered by intranasal aerosol.

As a result of challenging pigs, TMT group A (IN) had the highest average clinical score as well as the highest number of animal medical treatments. TMT group B (IN+IM) had the second highest average clinical score and the second highest number of individual animal treatments.

TMT group C given the commercial killed vaccine had the lowest average clinical score when compared to all TMT groups including the control group. No pigs in TMT group C required any individual medical treatments.

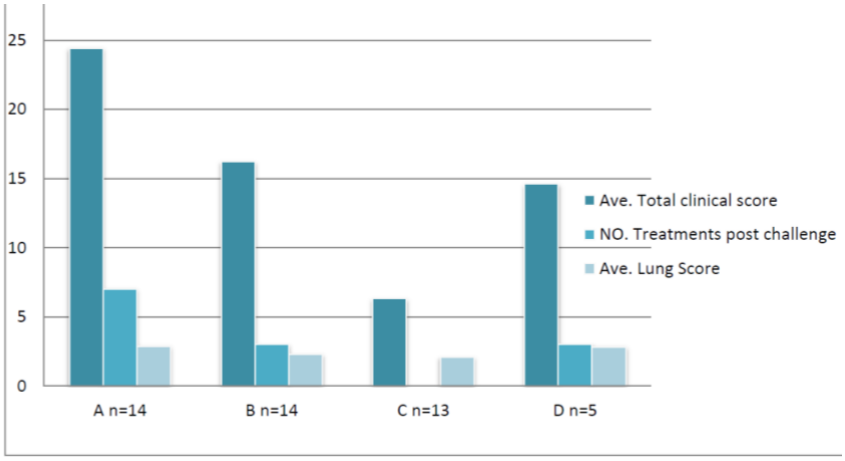

Figure 1: Level of disease expressed as clinical score, the number of antibiotic/anti-inflammatory treatments administered during a total of 30 observations conducted post challenge and average lung scores.

Lung score results showed that typical *Actinobacillus pleuropneumoniae* lesions were caused in some pigs in ALL treatment groups but pigs in the C group had superior immune protection not leading to clinical symptoms like fever, lethargy and coughing at the same degree than pigs in the other three treatment groups.

Ave. Lung Score as per Table 2 scoring system
TMT A 2.85
TMT B 2.29
TMT C 2.08
TMT D 2.80

0	healthy lung (no gross lesions noted)
1	< 10 % lung involved with localized congestion only
2	10% - 25% lung involved with consolidation or congestion only and no necrosis or pleurisy lesion
3	25% to 40% involved with some consolidation or congestion, mild pleurisy lesions and congestion
4	40 - 50% lung involved with some consolidation or congestion, mild pleurisy lesions and congestion
5	50% to 75% lung involved with some consolidation or congestion, pleurisy lesions and congestion
6	75% to 100% lung involvement with pleuropneumonia and consolidation, extensive pleural adhesions and necrotic lesions

Table 2: APP lung lesion scoring at slaughter

TMT	Ave. Pre-weanAB	5 day Post-wean AB	Ave. 63D antibody level S/P %	Average of 98 Day (Pre-challenge) antibody level S/P %	Average of 105 Day antibody level S/P %	Average of 112 Day antibody level S/P %	Average of APP antibody level S/P% at slaughter
A	58.35	59.11	10.59	6.80	20.06	69.00	95.17
B	47.26	49.77	7.78	8.73	24.42	66.89	78.96
C	35.94	36.19	5.54	42.68	73.72	113.31	128.72
D	38.04	46.24	7.30	8.04	18.48	49.22	68.52

Table 2 :APP15 Elisa screening antibody levels from weaning to slaughter

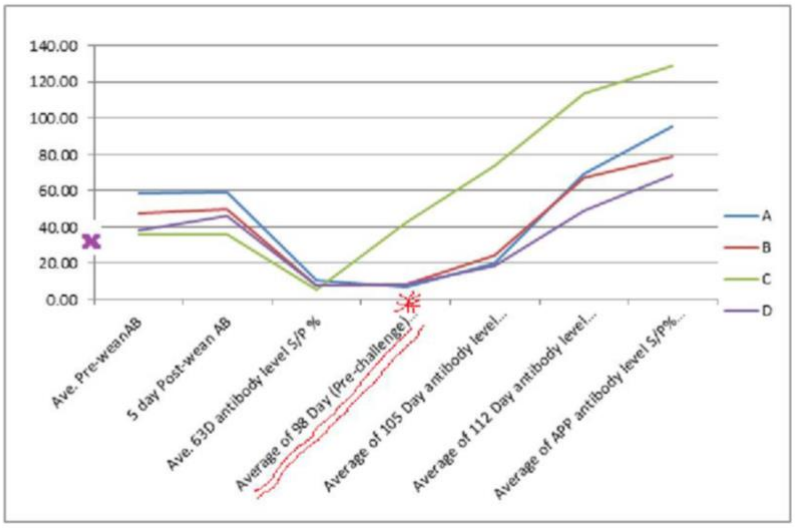

Figure 2: Illustrates the immune response of TMT C to killed autogenous vaccine at 63 and 84 days of age, all other treatments and the 5 control pigs failed to raise an immune response prior to challenge.
X = positive (protective) Antibody levels for the ELISA APP15 screening test, levels below S/P% of 30 are considered negative.

TMT C had the highest average antibody levels on pre-challenge samples and a corresponding low average clinical score with no medical treatments required in this group. Four individual pigs presented with antibody S/P% < 30 (negative test results).

The question what immune factors provided these pigs with superior immunity compared to the pigs in TMT Gp A and B which also presented with antibody S/P5 <30 but clinical signs were more pronounced and pigs required treatment in TMT A and B. Further work is required to determine factors that provide protective immunity. It is mentioned again here that TMT C was housed in the same size pen than the other treatment groups but with only 13 pigs. Did this provide reduced stress levels and therefore improved cellular and humoral response to the challenge?

4. Application of Research

The current commercial APP15 vaccine provided superior immune protection compared to the new vaccine.

5. Conclusion

The current commercial APP15 vaccine provided superior immune protection compared to the new vaccine.

6. Limitations/Risks

Risk that IM vaccine will cause adverse reaction to pigs. Vaccine technology should be further developed.

7. Recommendations

-Vaccine technology to be further developed.
-Current commercial vaccine and best practice management for APP should be used.
-Investigate Cell Mediated Immunity aspects of APP. Are colonised tonsils enough to establish immunity?
-Relationship of local immunity, humoral immunity to disease protection in commercial conditions
-Attenuated live APP vaccines requires re-evaluation for duration of presence in host and the time that is required to establish protective immunity
-Killed, autogenous vaccines provide good protection but three vaccinations and reduced host stressors are required.