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Salmonella control in layer chickens

A report for the Australian Egg Corporation Limited

by Dr Peter Groves

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Foreword

This project determined the capability to restrict Salmonella colonisation of and Salmonella shedding from layer chickens after differing vaccination regimes using live and inactivated Salmonella vaccines; and, to identify one or more efficacious and cost effective vaccination regime(s) to limit and achieve a reduction of Salmonella intestinal colonisation in commercial layer chickens in Australia.

This project was funded from industry revenue which is matched by funds provided by the Federal Government

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Dr Angus Crossan
Program Manager – R&D
Australian Egg Corporation Limited

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The Australian Egg Corporation Limited provided the funds which supported this project.

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Abbreviations

ARC	Australian Research Council
APVMA	Australian Pesticides and Veterinary Medicines Authority
BPW	Buffered peptone water
MKTTn	Muller Kaufman Tetrathionate Novobiocin
FSANZ	Food Standards Australia New Zealand
IgG	Immunoglobulin G
IGY	Egg yolk antibody
MSD	An international animal health company known as Merck in the USA
NWSFA	New South Wales Food Authority
NH&MRC	National Health and Medical Research Council
RMIT	Royal Melbourne Institute of Technology
SMID	Chromogenic medium for the selective isolation and differentiation of the genus *Salmonella* in food products (Biomerieux Australia)
WBC	White Blood Cell
XLD	Xylose Lysine Deoxycholate

Executive Summary

The objectives of this project were to determine the capability to restrict Salmonella colonisation of and Salmonella shedding from layer chickens after differing vaccination regimes using live and inactivated Salmonella vaccines; and, to identify one or more efficacious and cost effective vaccination regime(s) to limit and achieve a reduction of Salmonella intestinal colonisation in commercial layer chickens in Australia.

The vaccines studied in this project were the Australian Bioproperties Vaxsafe® Salmonella Typhimurium (ST) live vaccine, which has the aro-A gene deleted, and the Intervet-Schering Plough (now MSD) inactivated multivalent Salmonella vaccine (containing inactivated cultures of S. Typhimurium, S. Infantis, S. Montevideo and S. Zanzibar).

Phase 1 of the project evaluated nine different vaccination regimes against challenge at various ages in a commercial layer breed (Rhode Island Red x Rhode Island White Brown egg layers). In Phase 2 of this study, five vaccination programs were selected based on the outcome of work conducted in Phase 1 with the aim of evaluating the efficacy of each against infection by S. Typhimurium (serogroup B1), S. Infantis (serogroup C1) and S. Virchow (serogroup C1). The objective here was to confirm the efficacy against S. Typhimurium demonstrated in Phase 1 and to evaluate cross protection, if any, against Salmonella serovars, which were or were not represented in the inactivated vaccine.

In each phase, birds were vaccinated as per design and challenged at various ages (at 4, 12, 17, 25, 34, 52 and 56 weeks in Phase 1; and at 4, 10, 16 and 22 weeks in Phase 2). At each time point, birds were challenged with high doses (108 cfu/bird) orally with the appropriate serovars and maintained for three weeks before their caeca were removed aseptically and cultured for the presence of Salmonella. An ELISA (x-OvO) for anti-S. Typhimurium antibodies was used to evaluate response to vaccination and to look for any association between serological status and intestinal colonisation.

Phase 1 demonstrated that long lasting protection against S. Typhimurium colonisation requires injection of the vaccine (either live or inactivated). Oral administration of the attenuated live vaccine provided only short term protection. The best level of protection was achieved at about 16-17 weeks but some protection was afforded up to 34 weeks of age (using two injections of the live attenuated vaccine). It must be noted that the use of the live vaccine by subcutaneous injection is currently an off-label procedure and would require veterinary advice and supervision to be used.

Phase 2 evaluated several vaccination regimes employing injection. The efficacy of the two vaccines given by injection or the live (injected) followed by the inactivated vaccine was assessed. The use of the live vaccine by spray application to day-old birds followed by a live subcutaneous injection at six weeks was also assessed. Cross-protection was assessed using challenges with three different Salmonella enterica serovars. The most effective result was obtained with a combination of live (injected) followed by inactivated vaccines. This gave the best protection including greater cross-protection.

An adverse reaction was detected following subcutaneous injection of the live vaccine and this needs further research before strong recommendations for its use in this manner can be made.

Recommendations for the layer producer depending on his/her objectives have been produced. An association between serum ELISA titre for S. Typhimurium antibody and

protection was observed and it is recommended, regardless of vaccination regime, a mean antibody titre exceeding the minimum positive threshold for the ELISA (x-OvO) must be reached to be confident of sufficient enduring protection against colonisation of the gastrointestinal tract by Salmonella.

Further work to understand the observed adverse reaction and to establish the use of the live vaccine by a parenteral route, including dose rate, needs to be performed to determine the most efficacious use of these vaccines. Some preliminary work in this area is underway and will be reported in the near future.

Summary of important outcomes:
The use of the live aro-A deletion attenuated vaccine by the oral route provides only short term protection against caecal colonisation by Salmonella enterica serovars in the chicken (perhaps only three-four weeks at best).

Use of the live vaccine by injection provides rapid production of humoral anti-S. Typhimurium antibodies.

The inactivated vaccine elicits good antibody production but requires two vaccinations for good protection.

A mean serum antibody titre of at least 785 (log10 2.89) ELISA (x-OvO) units is required to ensure good protection against S. Typhimurium colonisation of the gastrointestinal tract of chickens. The higher the level achieved the better and the longer will be the protection provided.

The most effective regime used the live vaccine by subcutaneous injection followed by the inactivated vaccine by intramuscular injection. This also provided the best cross-protection against a serovar not included in either of the vaccines.
The injectible use of the live vaccine is an off-label procedure and was also associated with an adverse reaction in at least one breed of layer chicken. This finding needs further investigation.

The maintenance of a presence of Salmonella enterica serovars in chicken flocks relies on continual cyclic infection of susceptible chickens within the flock, as individual birds will rid themselves of an infection over time vaccines will usually prevent a disease but not infection and it is the latter that is desirable to protect public health. Even with an existing infection, by providing a level of immunological protection to the whole flock by vaccination, as represented by a desired level of humoral antibody, the likelihood of spread of salmonellae between susceptible birds is reduced and the long term result will be to assist in reduction, and possibly the eventual elimination, of salmonellae from the flock. Vaccination shows promise for the reduction of potential food borne infection from egg-producing flocks.

1 Introduction

Salmonella contamination of eggs and egg products is a major human health issue, both in reality and in perception for the Australian consumer and jurisdictional food safety bodies such as New South Wales Food Authority (NSWFA) and Food Standards Australia New Zealand (FSANZ). Food poisoning outbreaks in Australia have continued with an egg-borne source of infection sometimes incriminated. The egg industry is under continuous pressure both to improve and to be seen to be improving its approach to public health. Salmonellae are frequently isolated from the environment of poultry farms, sometimes involving the presence of serotypes which are regarded as serious for human health, in particular S. Typhimurium, which is the most frequent serovar isolated from human salmonellosis in Australia (28% of cases in 2009) and is also the most frequently detected serovar from Australian egg layer flocks (28.3% of isolates) (IMVS, 2009).

The genus Salmonella contains only two species, of which S. enterica deserves our interest here. S. enterica has six sub-species based on their O and H antigens. Subspecies I (S. enterica subsp. enterica) is our main interest and this subspecies includes over 2,300 serovars. These serovars are divided into numerous groups labelled A, B, C, D, E, etc. Of these, serogroup D includes the poultry specific serovars such as Pullorum, Gallinarum and Enteritidis. Serogroup A includes the human typhoid pathogen, serovar Typhi. Serogroup B includes serovars Typhimurium, Agona and Sofia, while serogroup C includes serovars Infantis, Montevideo, Virchow and Mbandaka. Serogroup E includes commonly found serovars such as Zanzibar, Orion, Tenessee and Senftenberg. Serogroups B and C represent the majority of serovars involved in human salmonellosis in Australia (IMVS, 2009).

Salmonella stands out as the most commonly reported microbiological agent responsible for food borne illness where eggs have been implicated as the cause. It has been estimated there are about 12,800 cases of egg-related salmonellosis per year in Australia, costing $44 million, and that the number of cases is rising (FSANZ, 2009).

Salmonellae are common members of the normal flora of many animals, including chickens, cattle and reptiles. The strains that cause human gastroenteritis are usually transmitted by chicken meat, eggs and dairy products (Engleberg et al., 2007). However, the mechanisms of pathogenicity of Salmonella infection are poorly understood. The transmission of Salmonella to humans by contaminated eggs has been a prominent international public health issue for more than two decades (Gast et al., 2011). Some of the major outbreaks of human salmonellosis have been associated with non poultry sources such as orange juice (Anon., 1999), peanut butter (Anon., 2009) and even non-food source such as contact with small turtles (Anon., 2012). Pires et al. (2010) however, have attributed 58% of human salmonellosis cases to which a source could be identified in Europe over 2005-2006, to egg products.

In Europe, following the S. Enteritidis problems of the last two decades, use of vaccination against Salmonella (originally aimed at S. Enteritidis but increasingly targeting other serotypes such as S. Typhimurium and S. Heidelberg) has become an essential tool in improving bacterial quality of table eggs and in gaining consumer confidence (Snow et al., 2007).

More recently, an inactivated Salmonella vaccine (Intervet) has been used with success by some Australian chicken meat companies in decreasing the prevalence of undesirable Salmonella serotypes in meat chicken breeder flocks (Groves & Pavic, 2006). This vaccine is an autogenous multivalent, containing organisms of three sero-groups (B, C & E) used

under Australian Pesticides and Veterinary Medicines Authority (APVMA) permit. The success with this autologous trivalent Salmonella vaccine has resulted in the incorporation of this vaccine into commercial broiler breeder production systems (Pavic et al., 2010).

A live mutant S. Typhimurium vaccine (Vaxsafe ST®, Bioproperties Australia - an Ar0-A deletion mutant of S. Typhimurium) has also been released for use in Australia. This live vaccine can be administered at day old or any other age and so help circumvent early infection. It is registered for oral (drinking water) application in chickens. It also could prove useful as a "primer" dose to later improve the immune response to the inactivated vaccine, making this more efficient or perhaps even allowing only a single dose to be required. The inactivated vaccine is expensive at present and this approach may enable the program to be more cost effective. Alternatively, the live vaccine may prove sufficiently effective alone.

This project was conducted over a two-year duration in response to the importance of this research for AECL.

Salmonella Enteritidis is not considered to be prevalent in Australian poultry flocks, which is of major concern to the food industry globally. S. Typhimurium is the most frequent serovar isolated from human salmonellosis in Australia (28% of cases in 2009) and is also the most frequently detected serovar from Australian egg layer flocks (28.3% of isolates) (IMVS, 2009). The Australian Salmonella Reference Centre (IMVS, 2009) reported that in 2009, S. Infantis was the second most commonly detected serovar from egg layers (after S. Typhimurium) and accounted for 2.1% of human infections. Cox et al. (2002) reported that Salmonella Infantis was the predominant Salmonella serovar in the Queensland egg industry.

2 Objectives

The objectives of this project were:

to determine the capability to reduce colonisation and shedding of Salmonella from, layer chickens after differing vaccination regimes using live and inactivated Salmonella vaccines;

and

to identify one or more efficacious and cost effective vaccination regime(s) to achieve an improvement in control of Salmonella caecal colonisation in commercial layer chickens in Australia.

3 Research Team

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Associate Investigator	Mrs Sue Sharpe	Birling Avian Laboratories; and University of NSW
Associate Investigator	Dr Wendy Muir	Poultry Research Foundation, The University of Sydney
Associate Supervisor	Prof. Julian Cox	University of NSW
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4 Methodology

4.1 Animal Ethics

All animal procedures undertaken in these studies were jointly approved by The University of Sydney Animal Ethics Committee (N00/8-2009/2/5144) and Birling Animal Ethics Committee (1038/12/10US). All procedures were carried out in accordance with the Animal Research Act of NSW (1985) and Regulations (2005) following the NH&MRC/ARC Code of Conduct (2007).

4.2 Procedures

The project adopted a two phase approach to the objectives:

4.2.1 Phase 1

Phase 1 involved establishing two flocks of commercial layer chickens, one reared in floor pens on deep litter, to simulate floor-reared operations, and one reared in cages and transferred to laying cages for the duration of the trial. The birds were given a number of vaccination programs involving the live and killed vaccines by various routes and at differing times. At various ages throughout the lives of these flocks (4, 12, 17, 25, 34, 52 and 56 weeks), birds were removed to experimental pens or cages and challenged with an oral dose of a field isolate of S. Typhimurium and held for three weeks. After this time these challenged birds were euthanized and their caeca aseptically collected and cultured for the presence of Salmonella.

At the conclusion of this phase, the most promising vaccine regimes identified were selected for confirmation against S. Typhimurium and for any ability to provide cross-protection against other Salmonella serovars.

4.2.2 Phase 1 Experimental Design

Nine vaccination regimes were studied in Phase 1 of the project (Table 4-1). Coding for this work uses the code for each vaccine (V or N) followed by the age in weeks at which it was administered (see Table 4-1). Birds were placed in floor pens on deep litter (new wood shavings) which were placed over plastic and covered the floor entirely. Each pen had two feed hoppers and one bell drinker. The facility was heated using a gas-fired space heater with a starting air temperature of 32oC and this was reduced by 1oC every second day until 21oC was reached at 21 days of age.

Vaxsafe® ST was given either by oral gavage using a stepper pipette (Finnpipettes®) using a 2.5ml tip set to deliver the desired 108 cfu dose in 0.25ml of sterile Phosphate Buffered Saline (PBS). Dilutions were performed assuming one vaccine 1000-dose vial contained a total of 1011 cfu. This was diluted into 250 ml PBS (equivalent to 108 per 0.25ml). When used as a subcutaneous injection, 0.25 ml of this dilution was administered using a separate 1ml syringe for each bird. Remaining diluted vaccine was returned to Birling Avian laboratories after each vaccination time and enumerated by adding 1 ml of the prepared vaccine into 9 ml of 0.1% peptone water and serial 1: 10 dilutions were made from this in 0.1% peptone water out to 10-8. Then duplicate aliquots of 0.1 ml of each dilution were placed onto SMID chromogenic Salmonella agar and spread using a sterile spreader stick. Plates were incubated at 37oC overnight and number of Salmonella colonies counted on appropriate dilutions to calculate cfu/ ml.

Table 4-1 Experimental Design for Phase 1 Studies

Group Code	Vaccination Regime	Flour Group	Cage Group
C	No vaccination - Control	Yes	Yes
V0 V3	Live1 vaccine at day old and 3 weeks	Yes	Yes
V0 V3 N12	Live[1] vaccine at day old and 3 weeks and Killed2 vaccine at 12 weeks	Yes	Yes
V0 V3 V6	Live[1] vaccine at day old, 3 and 6 weeks	Yes	Yes
N6 N12	Killed[2] vaccine at 12 weeks	Yes	Yes
V0 V3 N6 N12	Live[1] vaccine at day old and 3 weeks; Killed2 vaccine at 6 and 12 weeks	Yes	Yes
VS4 VS8[4]	Live[1] vaccine by subcutaneous injection3 at 4 and 8 weeks	Yes	No
V0 V4 V14	Live[1] vaccine at day old, 4 and 14 weeks	Yes	Yes

[1] Bioproperties Vaxsafe ST by oral gavage at 108 cfu/bird
[2] Intervet Salmonella vaccine at 0.5ml /bird by intramuscular injection
[3] Bioproperties Vaxsafe ST by subcutaneous injection at 108 cfu/bird
[4] Not used in the cage- reared group

The dose rate used at the outset was the original Bioproperties “single dose” rate (108 cfu/bird), but this was subsequently changed to 107 cfu/bird. To provide consistency, we continued to use the original dose rate.

The use of Vaxsafe ST by the injectible route (sub-cutaneous for group VS4 VS8 at 108 cfu/bird) is an off label usage and was included as an experimental variable in an attempt to find the most efficacious approach.

All nine vaccination regimes were used in the floor -reared birds while group 8 (VS4 VS8) was not included in the commercial cage- reared group (as this was an off-label use).

The cage -reared birds were housed in a commercial layer farm in Kemps Creek, New South Wales. The trial birds occupied one group of cages within a large commercial cage rearing shed and the birds were subject to normal rearing practices and management. Around two weeks of age, this flock was vaccinated against fowl pox and infectious bronchitis by a commercial vaccination crew. The date of this vaccination was changed at short notice and did not allow the investigators an opportunity to supervise this procedure. The intention was for the birds to move from cage to cage with vaccination, in a regular order so that all group identities were maintained. Only some birds in each pen had been tagged at this stage. It became obvious from checking wing tag numbers that this bird move was completely irregular. We could only identify several pens where we had confidence in the identity of all birds. This resulted in loss of useable numbers and the need to curtail challenge assessments at some ages from this group.

4.2.3 Phase 2

Phase 2 used vaccination regimes identified as promising in Phase 1.These were two doses of the killed vaccine, two doses of the live vaccine but administered by subcutaneous injection and a combination of the live vaccine by subcutaneous injection followed by a killed vaccine. One of the project collaborators also suggested that we evaluate the live vaccine administered by coarse spray at hatch and followed by a live vaccine injection at six weeks. These were compared with unvaccinated birds. These groups were reared in floor pens until the completion of the experiment and 12 birds per group were removed at 6, 10, 16 and 22 weeks of age and challenged separately with either S. Typhimurium, S. Infantis or S. Virchow. This attempted to confirm the Phase 1 results against S. Typhimurium and looked for cross-protection against another serovar that was also an antigen used in the killed vaccine (S. Infantis) and a serovar that was not represented in the killed vaccine (S. Virchow).

S. Infantis was chosen as a challenge serovar because it is included as an antigen in the multivalent killed vaccine used in this project and because of its relative importance within the egg layer industry and in human salmonellosis in Australia. S. Infantis belongs to serogroup C1.

4.2.4 Phase 2 Experimental Design

Vaccination regimes that looked the most promising from Phase 1 were selected for further study in Phase 2. The selected regimes are shown in Table 4-2. These included only injected vaccination approaches. One project co-operator requested that we also look at day old spray application of Vaxsafe® ST as a primer approach for a subsequent injectible vaccination, and this treatment was included.

Hy-Line Brown chicks were obtained for Phase 2 as they were guaranteed to be Salmonella free from the hatchery. All chicks were reared in separate pens at the

Zootechny floor pen facility in disinfected pens on new wood shavings. Commercial chick starter and pullet grower were purchased, free from any Salmonella inhibiting substances. Birds were vaccinated as described in the design (Table 4-2).

Table 4-2 - Experimental design for Phase 2 Studies

Group Code	Vaccination Regime
C	No vaccination - Control
N6 N12	Killed[2] vaccine at 6 and12 weeks
VS6 N12	Live1 vaccine by subcutaneous injection at 6 weeks and Killed[2] vaccine at 12 weeks
Vsp0 VS6	Live[1] vaccine by coarse spray at day old and live[1] vaccine by subcutaneous injection at 6 weeks
VS6 V018	Live[1] vaccine by subcutaneous injection at 6 weeks and live[1] vaccine by oral gavage at 18 weeks

[1] Bioproperties Vaxsafe ST at 108 cfu/bird
[2] Intervet Salmonella vaccine at 0.5ml /bird by intramuscular injection

4.2.5 Vaccines

Vaccines used in this project were:

4.2.5.1 Vaxsafe® ST manufactured by Bioproperties Australia

Vaxsafe® ST is a live attenuated (Ar0-A deletion mutant) Salmonella Typhimurium vaccine (Strain STM-1) developed at RMIT University in the early 1990s. This vaccine has only been used as a single oral or coarse spray application in layer pullets and broiler chickens in Australia in order to reduce colonisation by virulent Salmonella. The originally registered dose rate for Vaxsafe® ST was established as 10 8.0 cfu (colony forming units)/bird via the oral route in 2006. This was the dose rate employed in all studies in this project as this was the designated label rate at the start of the project. The dose rate was subsequently reduced to 10 7.0 cfu /bird in line with the dose of vaccine approved for use overseas (USA and Europe) for a comparable product manufactured by Fort Dodge Animal Health in the USA. This project began utilising the original dose rate and continued to do so to maintain consistency throughout. **We note that the use of this higher dose rate and also when given by subcutaneous injection is an off label use.**

This vaccine is coded as “V” in the treatment groups for this project. Batch number STM071421A was used throughout this study. The label stated that each vial held 1000 doses at >=108 cfu per dose. Shelf life of this batch, originally labelled as 23 Jan 2009, was extended by the manufacturer to 2012.

4.2.5.2 Intervet™ Inactivated Multivalent Salmonella vaccine

Intervet Australia (now MSD) was commissioned to produce an autologous multivalent inactivated vaccine using proprietary Selenvac® technology from Australian poultry field isolates. Strains are grown on iron-depleted agar, improving expression of the antigenic iiron rregulatory proteins (IRPs), stimulating the humoral response and increasing antibody titres, (Van Den Bosch, 2003). The multivalent vaccine was produced, using cell suspensions (3x108 cfu/ml). Serovars included in the vaccine were S. Typhimurium PT12 (belonging to serogroup B1), S. Infantis (serogroup C1), S. Montevideo (serogroup C1) and S. Zanzibar (serogroup E1). The vaccine contains thiomersal and formalin and Aluminium Hydroxide gel as adjuvant.

This vaccine is coded as “N” in the treatment groups for this project. Batch number 4078A-031 was used in Phase 1 and batch number B1016922 (expiry 3/11/2011) was used in the Phase 2 study. Label described this batch as containing 108 cells per ml. The vaccine was used under APVMA permit numbers 10434 (Phase 1) and 11924 (Phase 2).

4.2.6 Challenge Organisms

Challenge strains of S. Typhimurium phage type (PT) 108 or PT 135a (used in Phase 1 and Phase 2 experiments), S. Infantis and S. Virchow (used in Phase 2 experiment) were selected from recent poultry field isolations at Birling Avian Laboratories, Bringelly, NSW. Low passage cultures were used. These were stored on Cryovials at -80oC.

For each Salmonella strain, a bead from a Cryovial was incubated in buffered peptone water (BPW) (37ºC for 24 hours) and then transferred to Nutrient Agar (Oxoid, CM3) (for challenges up to 20 weeks of age and thereafter we used Nutrient Agar containing 2% mucin) and, post-incubation, a combined group of colonies were suspended in 0.9% peptone water to give a 75% transmittance (1.0 McFarland) in a BioMerieux 47100-00 DR 100 Colorimeter, equating to 2x108 cfu/ml. The number of cells was determined after administration by decimal dilution and spread plating on chromogenic Salmonella agar (SMID, BioMerieux).

4.2.7 Caecal culture and Salmonella detection

Three weeks following each of the challenges, the birds were humanly euthanized and their caeca were collected aseptically and cultured.

The Australian Standard AS 5013.10-2009 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella species, was used throughout.
The Salmonella detection method was:

Day 1: Pre-enrichment - : Make a 1:10 dilution of the faeces or caecum in buffered peptone water (BPW) and incubate at 36 +/- 1 oC overnight (16 – 20 hours).
Day 2: Prepare selective enrichment by transferring 1 ml of the pre-enrichment broth to 10 ml Muller Kaufman Tetrathionate Novobiocin enrichment broth (MKTTn) and 100 µl of the pre-enrichment broth into Rappaport-Vassiliadis Ssoy peptone (RVS). Incubate the MKTTn at 36.00C +/- 10C and the RVS at 41.50C +/- 0.50C overnight (18 – 24 hours).
Day 3: Spread a 10µl loop full from both broths are spread onto selective Xylose Lysine Deoxycholate (XLD) and Hektoen agar plates and these are incubated at 36.00C +/- 10C overnight (18 – 24 hours).
Day 4: Selection and subculture of suspect Salmonella colonies, by examining the XLD plate, whereby the typical Salmonella colonies have a slightly transparent red halo and a black centre, a pink-red zone may be seen in the media surrounding the colonies. Examine the Hektoen plates where the typical colonies appear black with greening of agar. Other Enterobacteria enterics typically appear yellow or green. Place a suspect colony onto a chromogenic Salmonella agar plate (Biomeriux SMID) for confirmation. Incubate overnight at 36.00C +/- 10C. Typical colonies are a mauve / purple colour.
Day 5: Place one suspect colony onto non-selective nutrient agar for biochemical confirmation and serotyping. Representative isolates were sent to the Australian Salmonella Reference Laboratory (IMVS, 2009) for confirmation.

4.2.8 Drag swabs

Sterile tampons were used to detect presence of salmonellae in the birds environment. A new tampon which did not contain bacterial inhibitory material was immersed in sterile BPW and then attached to a clean rod and dragged in a zig-zag pattern across the surface of the floor of the pen. The swab was then placed in a 250ml sterile jar containing sterile BPW and transported to the laboratory. This was treated as for the pre-enrichment sampl,e as described above and followed through the same detection procedure.

4.2.9 Statistical Analyses

The proportion of birds for which ST was isolated from the caeca was compared between each vaccinated group and the unvaccinated controls using contingency table analysis (Chi-square or Fisher's exact test if an expected cell value was less than five5) and this was performed using the Statcalc function of EpiInfoTM (Centers for Disease Control and Prevention, 2000). Quantitaive serology results from the ST ELISA were compared using ANOVA with means separated by Tukey's HSD test using a computerised statistics package (STATISTICA™ ver 6, Statsoft Inc, 2001, Tulsa OK, USA). Where ANOVA assumptions were not met (as measured by Levene's test for homogeneity of variance), the non-parametric Kruskal-Wallis ANOVA was used.

5 Results

5.1 Phase 1 Outcomes

Drag swabs taken from the floor- reared group showed no detectable presence of salmonellae in the environment for the duration of the study. However, samples from cages in the cage -reared facility detected the presence of S. Typhimurium from within the first few days of the birds' arrival. This may have compromised the outcome of the cage- reared group.

5.1.1 Challenge Outcomes

The results of the challenge experiments for Phase 1 are shown in Ttable 1.1. This includes results from both the floor- reared and cage- reared groups. Challenge studies utilised 8 birds per group until 20 weeks and 10 birds per group thereafter.

Table 5-1 Challenge outcomes from Phase 1 studies - Percent birds positive in caeca for S.Tymphimurium at various ages

Vaccination treatment	4 weeks[1] (n=8)	10 weeks[1] (n=8)	17 weeks[1] (n=8)	20 weeks[1] (n=10)	25 weeks[1] (n=10)	34 weeks[1] (n=10)	52 weeks[1] (n=10)	56 weeks[1] (n=12)
C	12.5	25.0	62.5	62.5	80.0	60.0	30.0	25.0
V0 V3	12.5	0.0	25.0	87.5	40.0	90.0	nt	nt
V0 V3 V12	nt	0.0	25.0	62.5	70.0	90.0	nt	nt
V0 V3 V6	nt	25.0	75.0	50.0	30.0	50.0	nt	nt
V0 V3 V6 N12	nt	12.5	25.0	50.0	40.0	60.0	10.0	25.0
V6 N12	nt	25.0	0.0*	37.5	50.0	60.0	40.0	41.7
V0 V3 N6 N12	nt	12.5	25.0	25.0	70.0	50.0	40.0	8.3
VS4 VS8	nt	25.0	0.0*	nt	30.0	10.0*	nt	58.3
V0 V4 V14	25.0	37.5	12.5	87.5	60.0	60.0	nt	nt
Difference from control	ns[3]	ns[3]	*P<0.05	ns[3]	ns[3]	*P<0.05	ns[3]	ns[3]

[1] Floor -reared group
[2] Cage- reared group
[3] Not significant P>0.05
nt - not tested

These results are shown individually by challenge time in Figures 5.1 to 5.8.

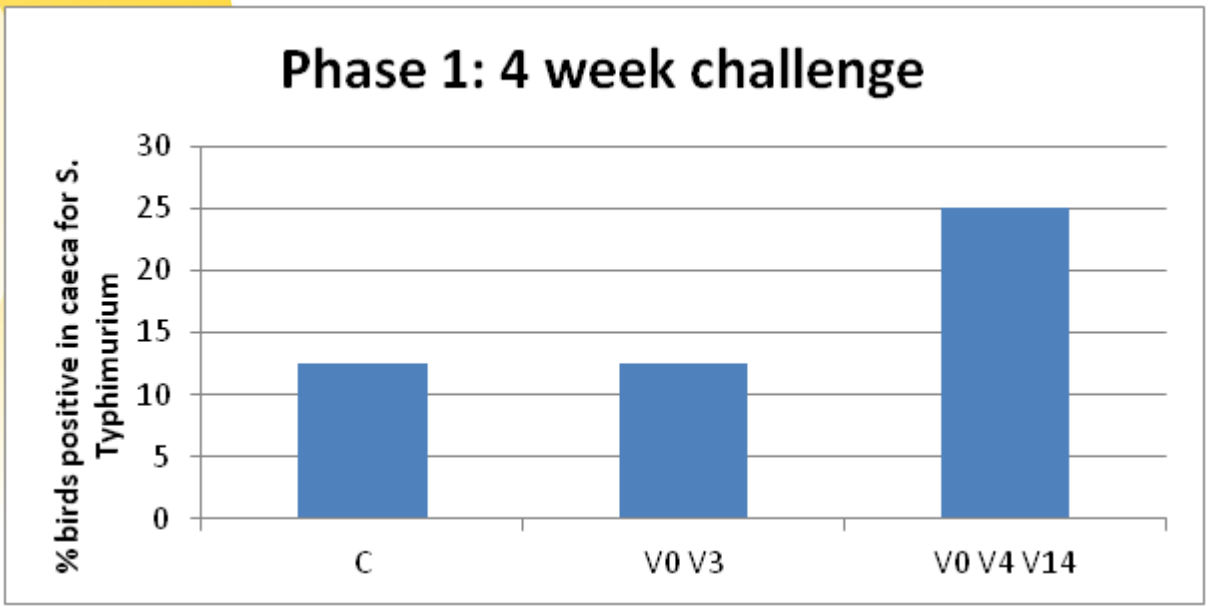

Figure 5-1 - 4 week challenge study - S. Tymphimurium PT 108

A challenge at 4 weeks was attempted with groups that had received vaccines at day 0. No protection was demonstrable with oral use of Vaxsafe® ST at this time. This was complicated as only one control bird (out of eight) became colonised.

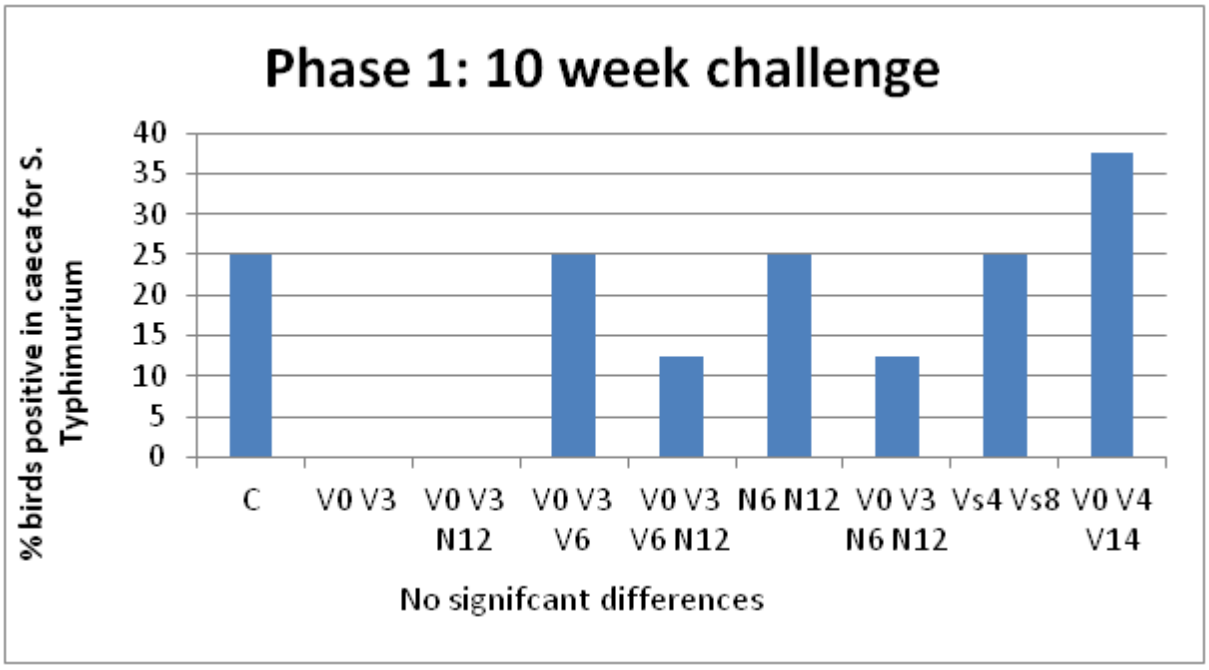

Figure 5-2 - 10 week challenge study - S.Tymphimurium PT 108

At 10 weeks of age all groups had received at least one vaccination. Only 25% of the control birds showed detectable colonisation at this age. There was no detectable protection provided by any combination of live oral vaccine (even when given up to three times). A single injection of either Vaxsafe® ST or the Intervet inactivated vaccine did not produce significant decreases in colonisation.

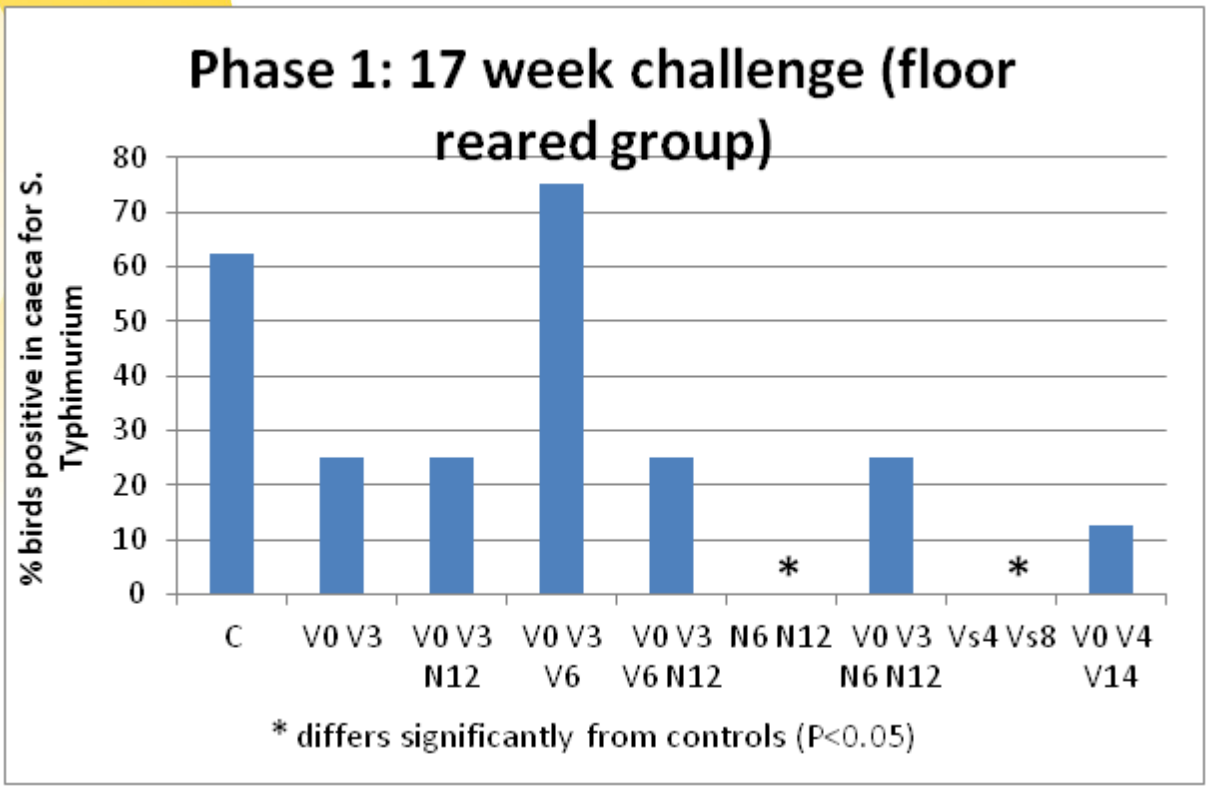

Figure 5-3 - 17 week challenge with S. Tymphimurium PT 108

By 17 weeks, all vaccination regimes had been completed. Five out of eight control birds were successfully colonised by the challenge organism at this age. While there appeared to have been some reduction in colonisation from several groups, only the groups receiving dual injection of either two inactivated vaccines or two live vaccines showed a significant reduction. The group which had received vaccination with live vaccine orally (V0 V4 V14) gave the next most promising result, noting that the last live oral dose was quite recent (14 weeks).

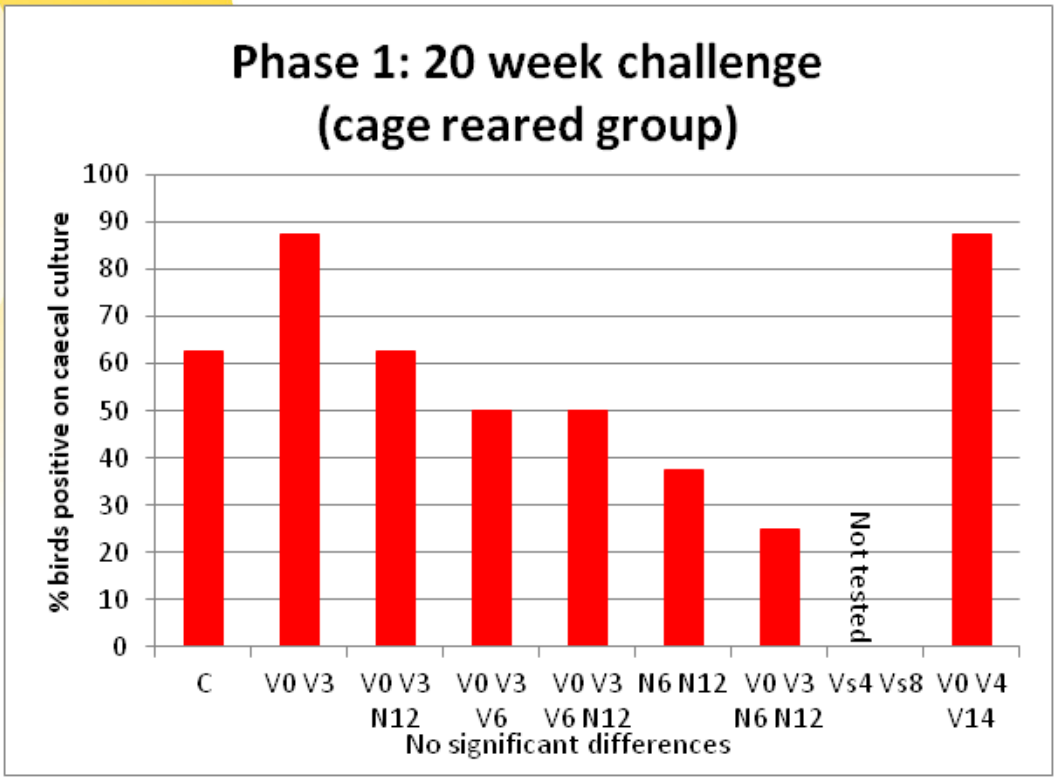

Figure 5-4 - 20 week challenge with S. Tymphimurium PT 135a

Due to the loss of a number of birds from the cage -reared group (as described above), only a small number of challenges were possible and these were carried out at the more crucial times in this phase of the project. At 20 weeks, birds from the cage -reared group were challenged. Five out of eight controls were successfully colonised. We could not demonstrate sufficient reduction in colonisation with any group to give statistical significance, although there appeared to be some reduction by groups receiving two inactivated injections (note,: the live injectible treatment was not used in the cage reared group).

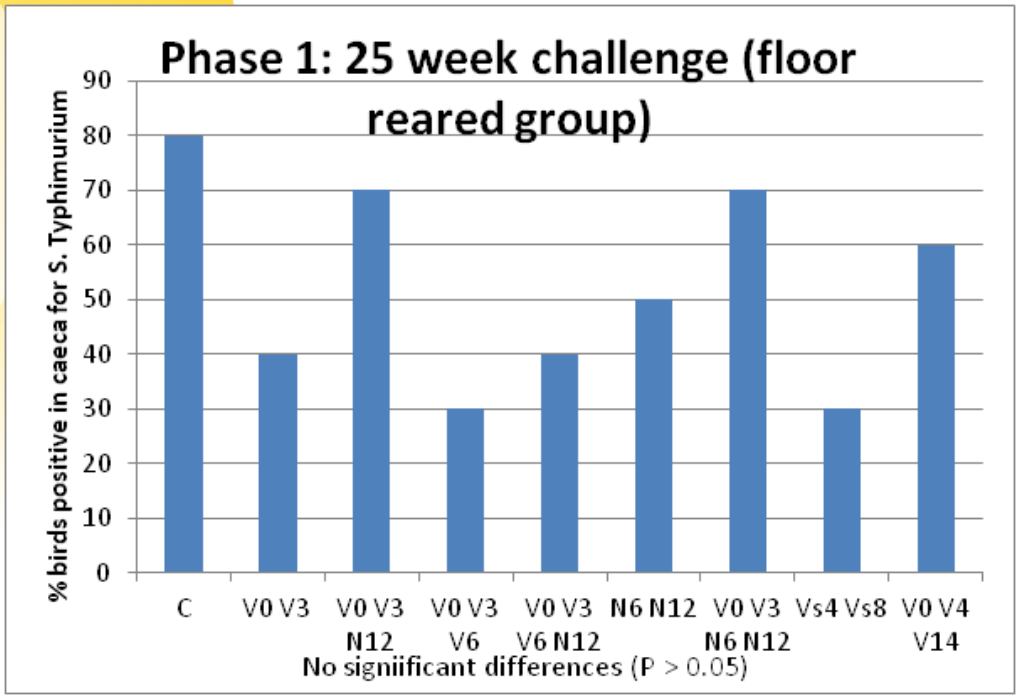

Figure 5-5 - 25 week challenge with S. Tymphimurium PT 108

At 25 weeks, 8 out of 10 controls were successfully colonised by the challenge organism, (using mucin in the challenge culture procedure). There were no statistically significant reductions in colonisation although treatments V0 V3 V6 and VS4 VS8 approached this (P= 0.07).

At 34 weeks, floor- reared birds were again challenged, using mucin-exposed cultures of S. Typhimurium. 60% of the controls were successfully colonised. Only the Vs4 Vs8 (live vaccine by s/c injection) maintained a significant reduction in colonisation compared with the control group at this age.

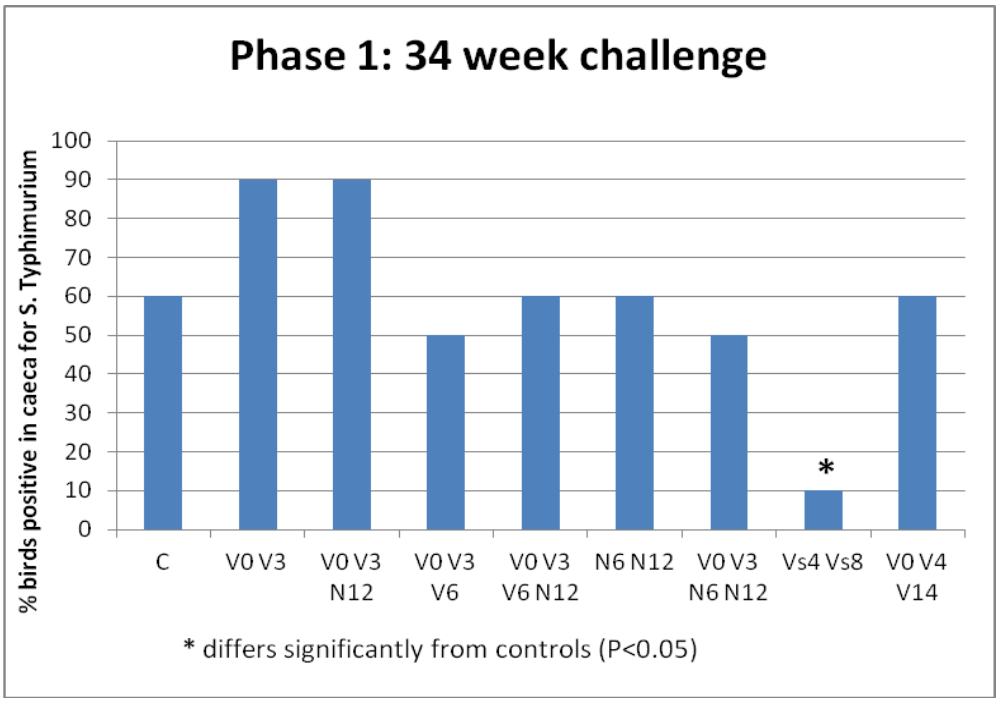

Figure 5-6 - 34 week challenge with S. Typhimurium PT108

Following this challenge, only vaccinated groups that had shown some earlier promise were used for further study. This included the following groups: V0 V3 V6 N12; N6 N12; V0 V3 N6 N12; and, Vs4 Vs8 (floor-reared group only).

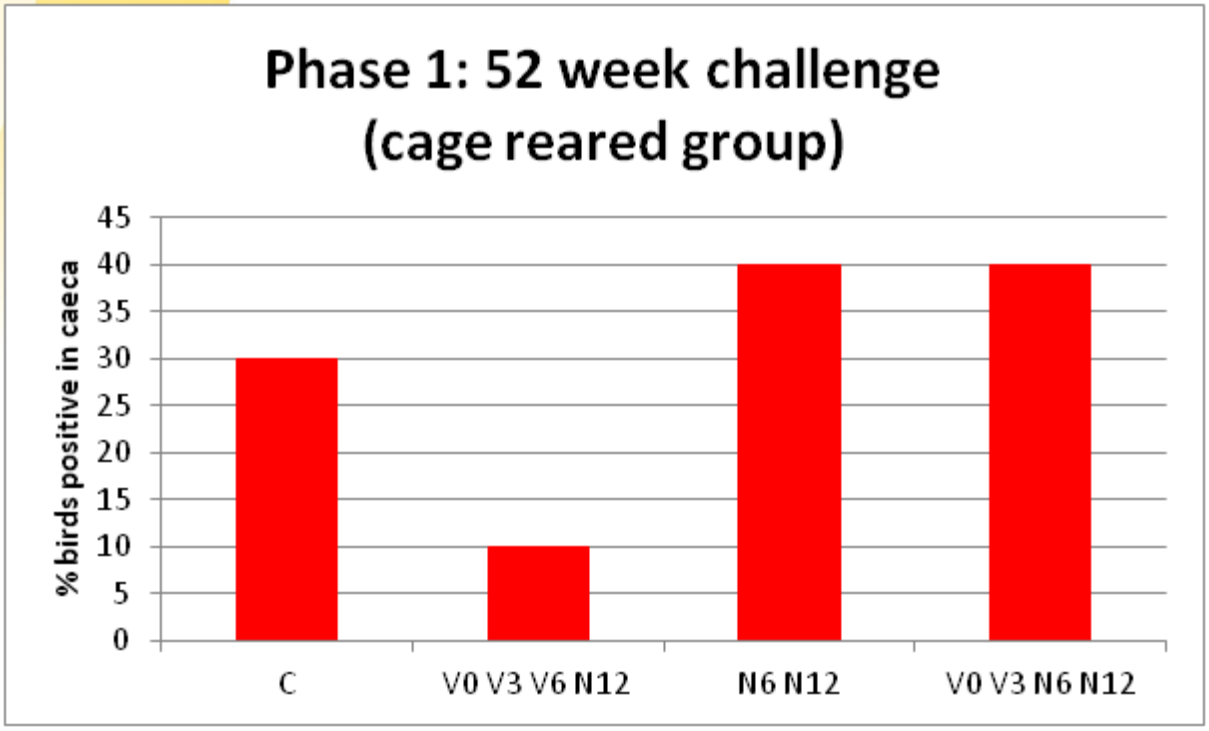

Figure 5-7 - 52 week challenge with S. Tymphimurium PT 108

At 52 weeks, the remaining cage -reared group birds were challenged. Only 30% of the control group was colonised and there were no significant differences in colonisation seen with any of the vaccinated groups studied at this age.

At 56 weeks, the floor reared birds in these groups were also challenged, with 25% of the controls successfully colonised and with similar non-different results, including that from the s/c injected live vaccine group.

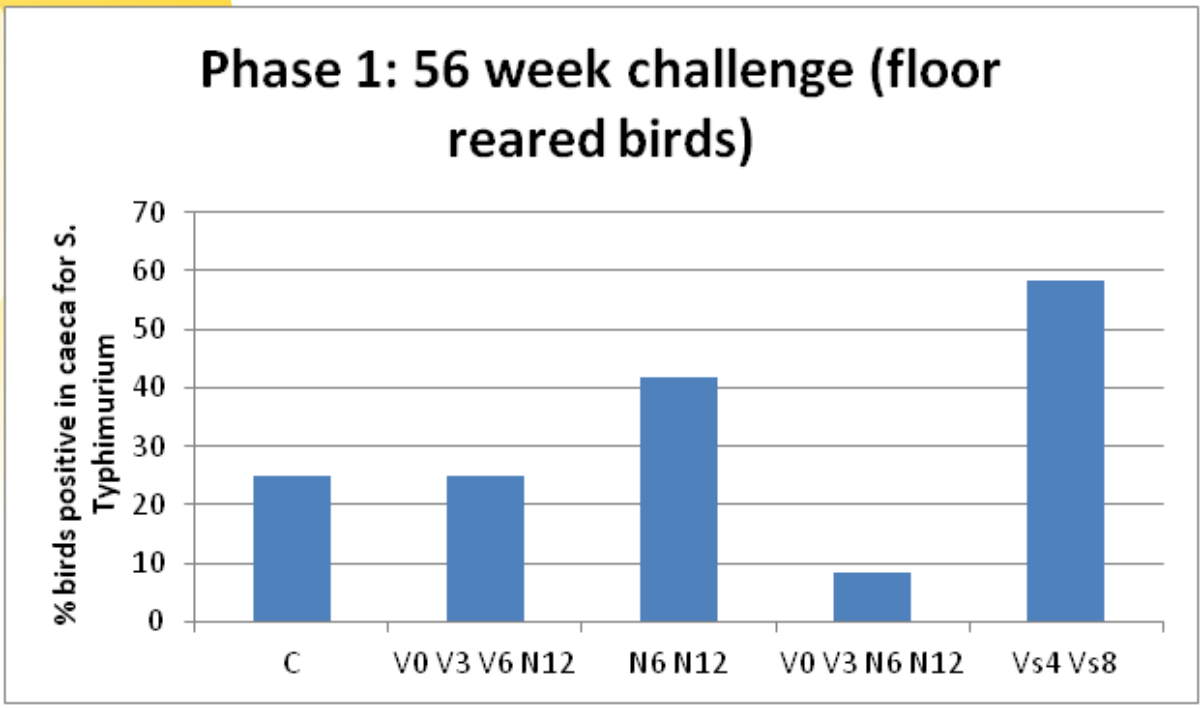

Figure 5-8 - 56 week challenge with S. Tymphimurium PT 108

5.1.2 Serological Outcomes

Results of continued serological analysis of anti-S . Typhimurium antibodies in each vaccine group over time is shown in Figure 5.9.

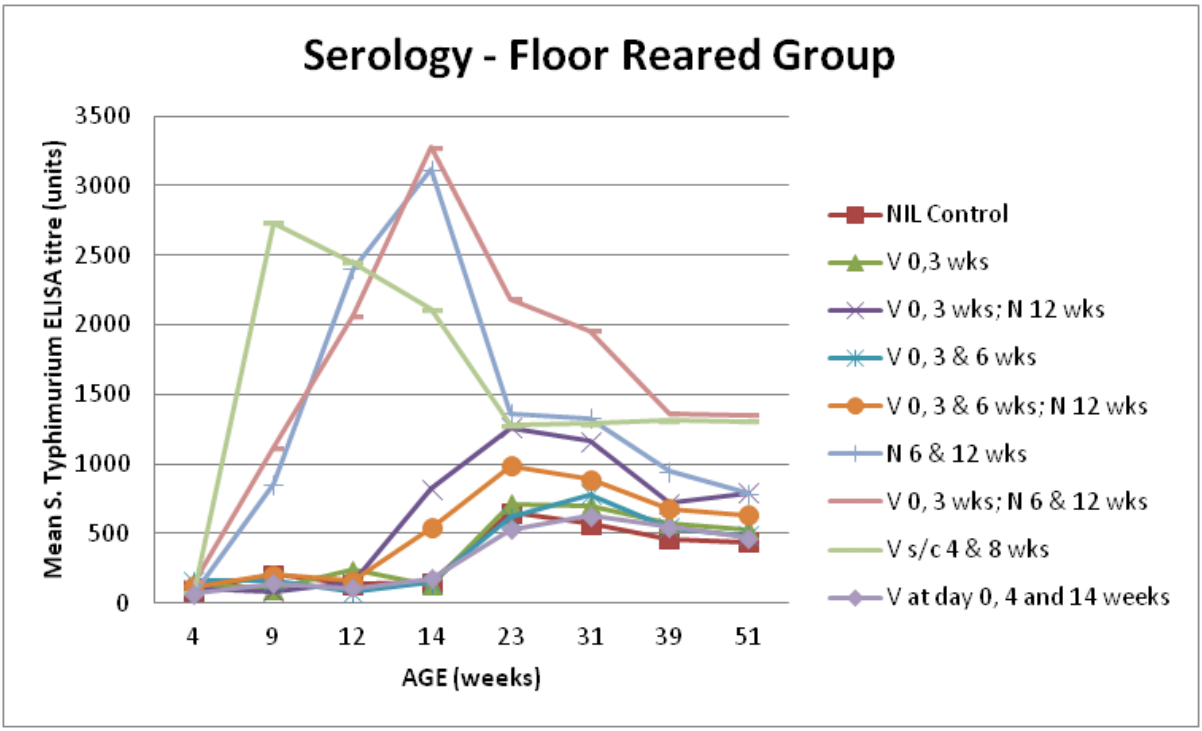

Figure 5-9 - Serological change in anti-S. Tymphimurium antibody following vaccination

The non-vaccinated control group showed no seroconversion over the study period, although there was a rise in titre level below the “positive” threshold from 23 weeks onwards. This corresponded with the move to the layer facility at University of Sydney and

with the onset of lay, and may reflect a non-specific response. All of the vaccinated groups which received only the live vaccine by the oral route showed an identical pattern to the controls in serological response. Groups which received only a single inactivated dose at 12 weeks of age (V0 V3 N12 and V0 V3 V6 N12) showed a serological rise above a "positive" level after 14 weeks, rising to a moderate peak by 23 weeks and then declining after 31 weeks to a "sub-positive" titre. After an inactivated vaccination at six weeks (V0 V3 N6 N12; and N6 N12), titres rose into the positive field by nine weeks of age and to high levels by 12 weeks. After a second inactivated vaccination at 12 weeks, titres for these two groups peaked at 14 weeks of age at very high levels and subsequently declined. The group receiving the live vaccine by subcutaneous injection at four and eight weeks showed a rapid high rise in serological titre by nine weeks, followed by a subsequent decline which still maintained high levels late into productive life. It became somewhat apparent that only the groups which achieved a serum titre above the ELISA test's positive cut off (725 ELISA units) that were able to demonstrate significant and sustained protection against some level of S. Typhimurium colonisation.

5.2 Selection of vaccination regimes for Phase 2

It became obvious that achieving colonisation with S. Typhimurium in this breed of layer chicken (Rhode Island breed) was difficult, especially as the bird aged. This was expected from the literature (see section 6 Discussion) but was more difficult than our previous experience with meat breeds (Groves et al., 2006; Pavic et al., 2010). Despite this, it could be observed that the live vaccine when given orally did not provide sustained protection (possibly only three-four weeks at best), even following repeated administration. However the administration of this vaccine by subcutaneous injection provided effective and prolonged protection. Only the injected vaccines gave sustained protection at any level in Phase 1 studies, and only if given twice. Hence the dually injection-vaccinated groups were selected for Phase 2 studies. It also looked valuable to assess the possible effects from a combined use of the live and killed vaccines by injection which may provide a stronger result than with either type alone. Thus the groups selected as shown in Table M2 were devised. One of the project co-operators requested that we also look at the live vaccine administered by coarse aerosol spray at hatch, as this had become a registered route of administration and was being utilised by segments of the industry. It was also intended to repeat the two s/c live vaccine group but due to unforseen issues with bird reactions (Groves and Sharpe, 2012), this regime had to be modified as shown in group Vs6 Vo18 in Table 4-2.

5.3 Phase 2 Outcomes

Drag swab samples taken in each pen of the rearing facility were originally negative but at three weeks of age the environmental presence of Salmonella serovars was detected in three pens of the facility (see Appendix A). The presence of these organisms remained for a few weeks and then became undetectable. The salmonellae were not detected in any other pen at any stage subsequently. Drag swabs are an extremely sensitive method of salmonellae detection, especially within small pens. The affected pens were not used in any further studies in this project and it is confidently believed that this limited contamination did not compromise the further assessments conducted within this study.

5.3.1 Challenge outcomes – Phase 2

Blood samples were collected more extensively in Phase 2, following the interesting serological picture which emerged from the Phase 1 study.

The Vsp0 VS6 group displayed several birds with positive serological titres to S. Typhimurium after the initial spray vaccination, at variance to that seen following oral inoculation. An early challenge for birds from this group compared with control birds only using only S. Typhimurium challenge was included at six weeks of age, to assess whether this would provide early protection. The results are shown in Table 5-2. Following this, all vaccine groups were challenged separately with S. Typhimurium, S. Infantis or S. Virchow at 10, 16 and 22 weeks of age. Results from these challenges are shown in Tables 5-3, 5-4 and 5-5 respectively. The ages chosen were aimed at describing protection against challenge, four weeks following a single vaccination (10 weeks), four weeks following the second vaccine application (16 weeks) and at point of lay (22 weeks). All challenges in Phase 2 involved 12 birds per group.

Table 5-2 - Phase 2 S. Typhimurium challenge at six weeks

Vaccine group	Vaccination applied prior to challenge[1]	Percent caeca positive three weeks post challenge
Control	Nil	41.7%
Vsp0 VS6	Vaxsafe® ST day old spray	58.3%
P = (difference to control)		*0.41*

[1]Birds had received only the first vaccination prior to their challenge.

There was no demonstrable protection against S. Typhimurium colonisation of caeca afforded by the spray vaccination at day old by six weeks of age.

Table 5-3 - Phase 2 Challenge at 10 weeks of age

Vaccine group	Vaccination applied prior to challenge[1]	S. Tymphimurium % caeca positive	S. Infantis % caeca positive	S. Virchow % caeca positive
C	Nil	50.0	58.3	50.0
N6 N12	N6	25.0	25.0	16.7
VS6 N12 & VS6 Vo18[2]	VS6	25.0	58.3	16.7
Vsp0 VS6	V0 VS6	16.7	16.7	16.7
P[3] =		*0.30*	*0.07*	*0.15*

[1]Only vaccines applied up to six weeks of age
[2] These groups were vaccinated identically to this age
[3]Chi-sq or Fisher's exact test if an expected cell value <5

Table 5-4 - Phase 2 Challenge outcomes at 16 weeks of age

Vaccine group[1]	S. Tymphimurium % caeca positive	S. Infantis % caeca positive	S. Virchow % caeca positive
C	83.3[a]	91.7[a]	91.7
N6 N12	66.7[a]	41.7[bc]	75.0
VS6 N12	25.0[b]	8.3[c]	50.0[3]
Vsp0 VS6	33.3[b]	66.7[ab]	66.7
VS6 Vo18[1]	Not done	Not done	Not done
P[2] =	*0.012*	*0.0004*	*0.15*

[abc] Means with different superscripts differ significantly (P <0.05)
[1] Group VS6 Vo18 was not included in this challenge due to the incomplete vaccine regime at this age (see Materials and Methods for explanation).
[2] Chi-sq or Fisher's exact test if an expected cell value <5
[3] P= Probability different from control group by chance

Table 5-5 - Phase 2 Challenge outcomes at 22 weeks of age

Vaccine group	S. Tymphimurium % caeca positive	S. Infantis % caeca positive	S. Virchow % caeca positive
C	23.1	91.7[a]	83.3[a]
N6 N12	14.3	21.4[b]	85.7[s]
VS6 N12	8.3	8.3[b]	33.3[b]
Vsp0 VS6	0.0	30.8[b]	53.9[ab]
VS6 Vo18[1]	7.1	33.3[b]	57.1[ab]
P [1]=	*0.39*	*0.0003*	*0.034*

[ab] Means with different superscripts differ significantly (P <0.05)
[1] Probability different from control by chance

The outcomes are considered below under graphs for each species challenge (Figures 5-10, 5-11 and 5-12).

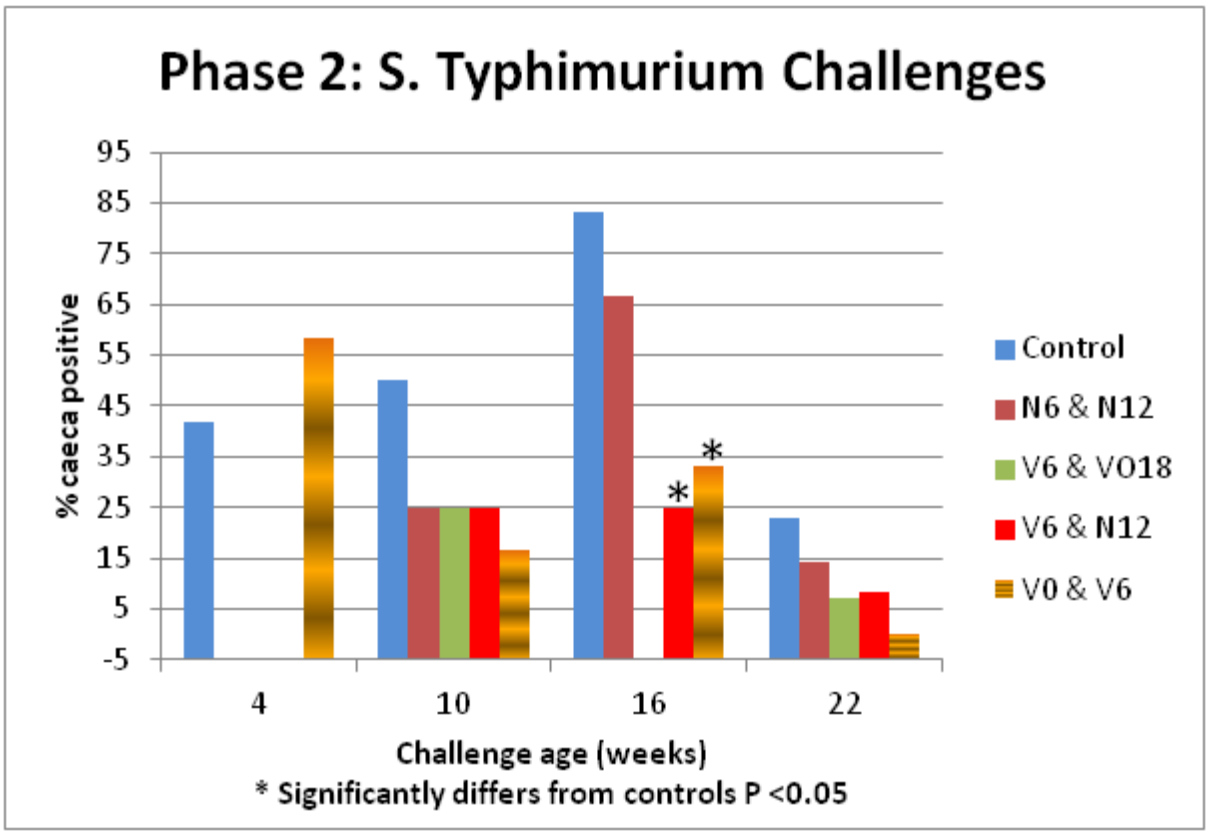

Figure 5-10 – *S*. Typhimurium challenge results by age in Phase 2

At ages up to 16 weeks, challenge with S. Typhimurium resulted in high “take” levels for the control group. This gave considerable ability to detect a reduction in caecal colonisation due to the vaccine effect. At 10 weeks, although there were lower numbers of birds colonised in each vaccine group, this was not significantly different to the control group. Hence the protection provided by single application of either the live or inactivated vaccine was insufficient for significant protection against caecal colonisation by S. Typhimurium. At 16 weeks, following the completion of two vaccine applications, the group which had received a live vaccine at six weeks by injection followed by a killed vaccine at 12 weeks had significantly reduced colonisation after challenge with S. Typhimurium. The group which had received live vaccine by spray at a day old, followed by a live vaccine by injection at six weeks also gave significant protection at 16 weeks. The group which

received two inactivated vaccines did not give significant protection at 16 weeks in Phase 2, which is at variance with the results from Phase 1.

At 22 weeks the ability to colonise the control birds with S. Typhimurium was much reduced, achieving only 23% positive caeca. Although the colonisation of the vaccinated groups was even lower, we could not demonstrate a significant reduction against this low result in the controls with any vaccine group.

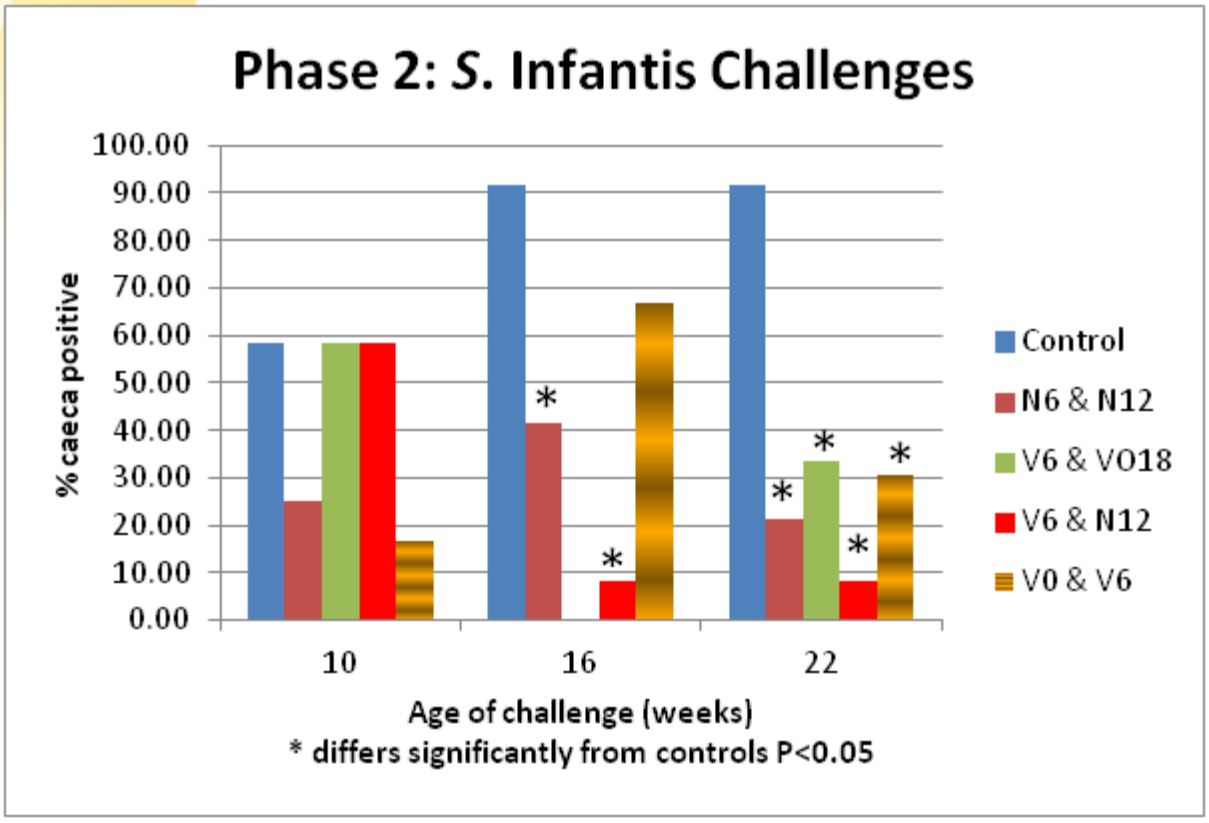

Figure 5-11 *S*. Infantis challenge results by age in Phase 2

High levels of colonisation of the control birds were achieved at each age with S. Infantis. Again, a single dose of either vaccine was unable to provide significant protection against S. Infantis challenge at 10 weeks. After two injectible doses of either the killed or live followed by killed injection gave significant protection against caecal colonisation by S. Infantis at 16 weeks. At 22 weeks, both dual injected vaccine groups (N6, N12 and V6 N12), the groups which received live vaccine by injection at six weeks and orally at 18 weeks and the group given day old spray vaccine at day old followed by injection of live vaccine at 6 weeks all significantly reduced S. Infantis colonisation.

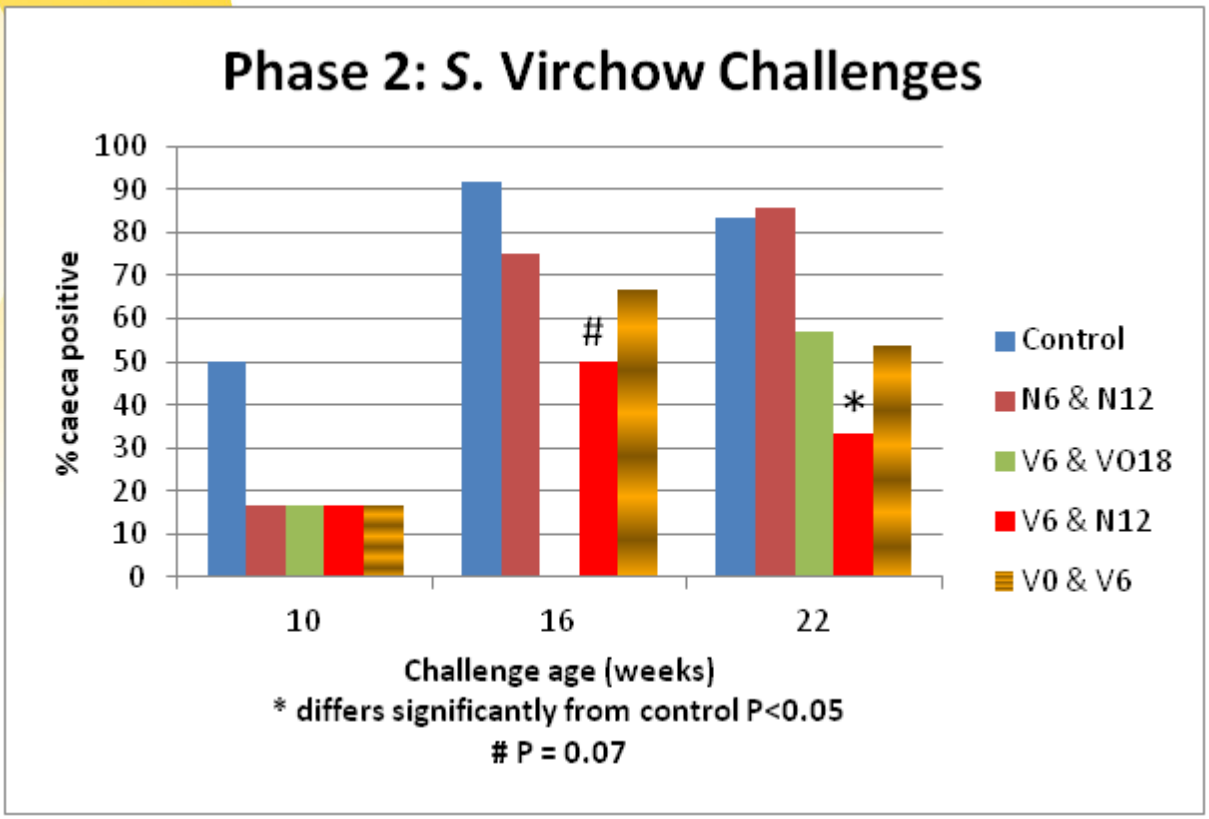

Figure 5-12 *S*. Virchow challenge results by age in Phase 2

Effective colonisation of the control birds with S. Virchow was achieved at all ages in this study. As with the other serovars, no significant reduction in caecal colonisation with S. Virchow after a single vaccine administration was demonstrated at 10 weeks. S. Virchow is not represented in the killed vaccine. At 16 weeks, no protection against S. Virchow caecal colonisation was seen with either the killed vaccine the spray followed by injected live vaccine applications, however the combination of a live injected vaccine at six weeks, followed by a killed vaccine at 12 weeks showed a decrease in caecal colonisation which approached significance (P = 0.07). At 22 weeks this was the only group (V6 N12) to provide a significant reduction in caecal colonisation with S. Virchow.

5.3.2 Serological outcomes – Phase 2

All vaccination programs in Phase 2 and each of the experimental S. Typhimurium challenges induced significant (P < 0.05) seroconversion compared to the unvaccinated birds, as measured by the ELISA (x-OvO). All S. Typhimurium challenged birds showed a substantial humoral immune response, by increased antibody titres in all groups, including the unvaccinated controls. The post-challenge serum titre increase seen in the control birds was similar to that achieved from administration of either of the vaccines by parenteral routes. Interestingly the results shown in Figure 5-13, the injected live vaccine at six weeks and inactivated vaccine at 12 weeks increased to a higher and longer lasting level of antibodies than the other three groups. These included Group 2, the killed multivalent autogenous vaccine at six and 12 weeks. The S. Infantis and S. Virchow challenged birds did not show increased level of S. Typhimurium serum antibodies, which supports the specificity of the ELISA test.

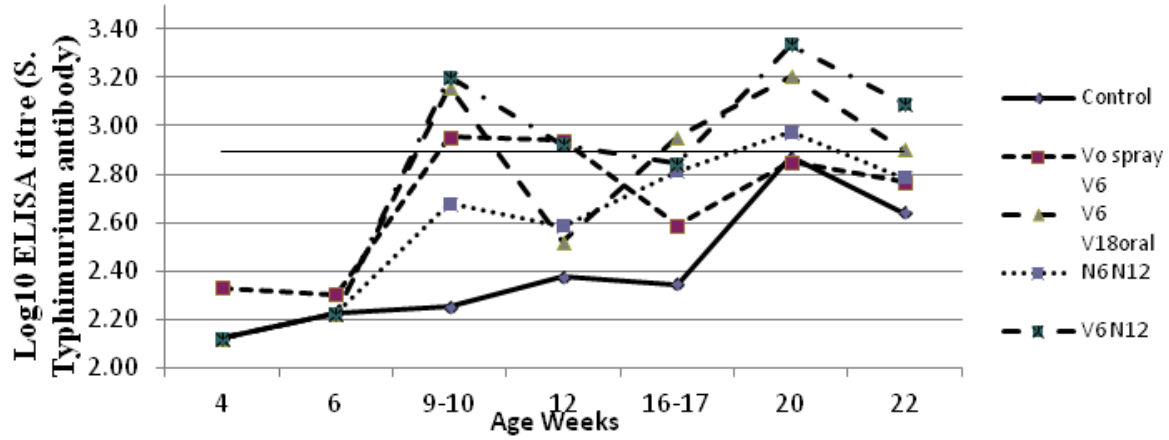

Figure 5-13 - Vaccinated birds: Serological results: 4-22 weeks

Antibody titres in experimentally unchallenged birds did not appreciably change by 13 weeks and remained below the cut-off positive point for the entire trial, as shown in Figure 2.4. Antibody response was detected by three weeks following injection of either vaccine. The humoral antibody response to the subcutaneous injection of the live vaccine exceeded that from the killed vaccine. A single live vaccination caused titres to exceed the threshold level of the ELISA test ((positive titre = >785 units or log10 2.895 as shown in Figure 5-13) after a single injection while the killed vaccine required two applications to achieve this result). The titre rise seen in the N6 N12 group was much lower in Phase 2 than was achieved in Phase 1 and, even following the second application, failed to rise above the threshold titre for positive until 20 weeks and declined below this by 22 weeks (compare Figures 5-9 and 5-13). This may help explain the poor challenge results at 16 weeks with this group. Interestingly the V6 Vo18 group showed a marked increase in titre following the oral administration of live vaccine in birds that had previously received live vaccine by injection.

At 10 weeks of age most of the birds had only received one vaccination (at six6 weeks) and the resulting changes in serology comparing challenged and unchallenged birds are shown in Figure 5-14.

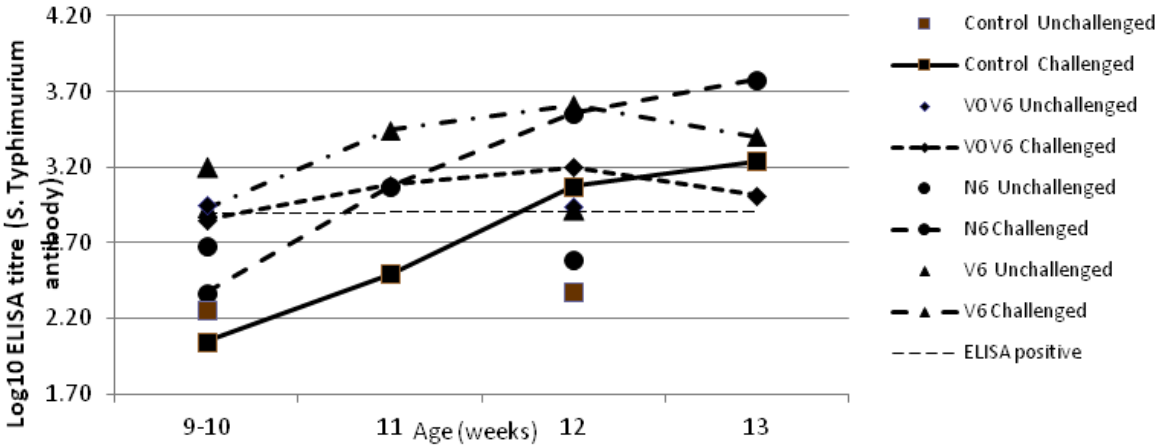

Figure 5-14 - Challenge Study: 10 week serological results. Log_{10} ST ELISA titre

Following the S. Typhimurium challenge at 10 weeks, the birds that had received the live vaccine at six weeks showed an increase in titre over two weeks and then an apparent decline by the third week. In contrast the inactivated vaccinated and control birds showed a marked increase in serum antibody titre that continued to rise by the third week.
By 16 weeks, following two vaccinations the results indicated that birds with elevated S. Typhimurium serum antibody levels had a decreased amount of Salmonella colonisation. The vaccination program that was significantly effective in providing protection was V6 N12, the live vaccinated at six weeks, followed by the killed vaccine at 12 weeks.

In Phase 2, every bird that was challenged with S. Typhimurium was blood sampled prior to and after challenge. There appears to be an association between Salmonella Typhimurium ELISA titres greater than 785 ELISA units (log_{10} 2.89) and protection against caecal colonisation. Table 5-6 shows a comparison of the birds' caecal colonisation with S. Typhimurium with their pre-challenge ELISA titre during the 16 week challenge study and this is depicted in Figure 5-14.

Table 5-6 - Comparison of pre-challenge S. Typhimurium ELISA titres at 16 weeks and caecal culture results following challenge after three weeks

Caecal culture result at 19 weeks	Mean Log_{10} ELISA titre for S. Tm at 16 weeks	95% confidence intervals of the mean log_{10} titre	Lower quartile titre	Median titre	Upper quartile titre
Positive (n=25)	2.23^{b}	1.88-2.57	2.17	2.42	2.61
Negative (n=23)	2.85^{b}	2.46-3.24	2.50	2.84	3.48
P=	*0.02*				

^{ab} means with different superscripts differ significantly (Student's t-test, P < 0.05)

This is a strong indication that serum anti-S. Typhimurium antibody levels are associated with reduced caecal colonisation with S. Typhimurium. Note that the mean and median titres for birds which returned negative caecal culture results was very close to the threshold level for a positive serological titre in the ELISA (x-OvO) (2.85 compared to 2.89 log_{10} respectively) and the upper 95% confidence limit for the mean titre for birds which returned a positive caecal culture was below this value.

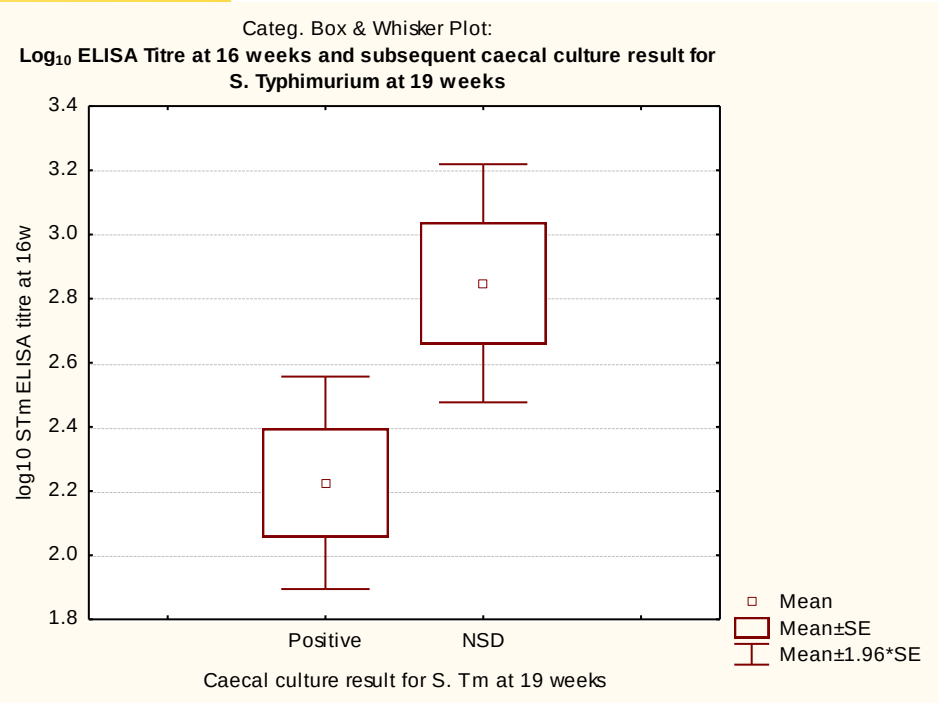

Figure 5-15 –*S*. Typhimurium antibody titre at 16 weeks grouped by their subsequent caecal culture result at 19 weeks

Interestingly, if birds challenged with any of the three Salmonella serovars are combined into a single analysis for the 16 week challenge, the mean titre for birds showing positive caecal culture results is $\log_{10}$ 2.41 while for those returning a negative caecal culture the mean titre was $\log_{10}$ 2.90 (significantly different, P = 0.002).

5.3.2.1 Egg yolk antibody study

The egg yolk antibody (IgY) levels correlated well with the blood results, presented previously. The inactivated vaccination Groups 6 and 7 achieved the highest antibody level, which was decreasing at 50 weeks. The live vaccine given orally showed only short term protection.

Results are summarised in Figures 5-16 and 5-17 for the floor -reared and cage -reared groups respectively.

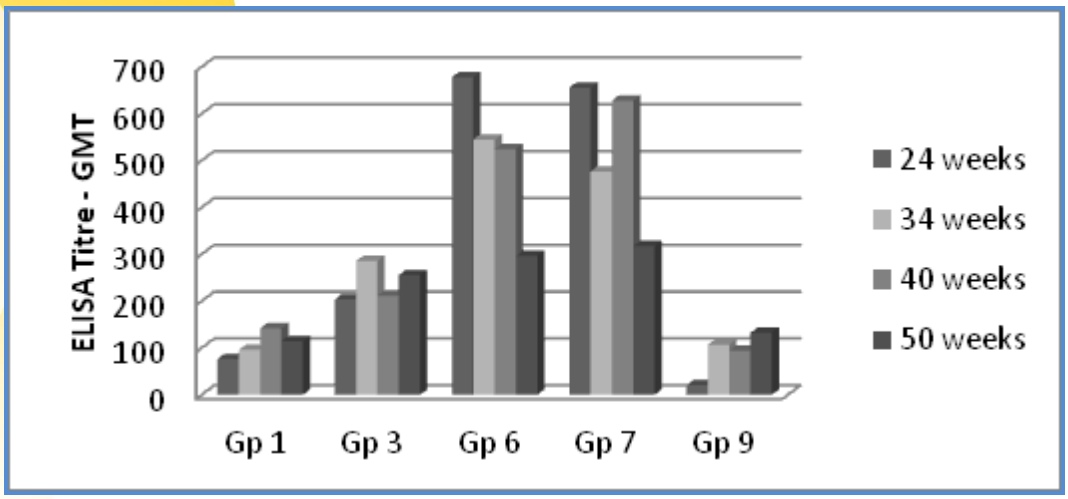

Figure 5-16 - Floor Reared - Egg Yolk IgY Antibody Levels

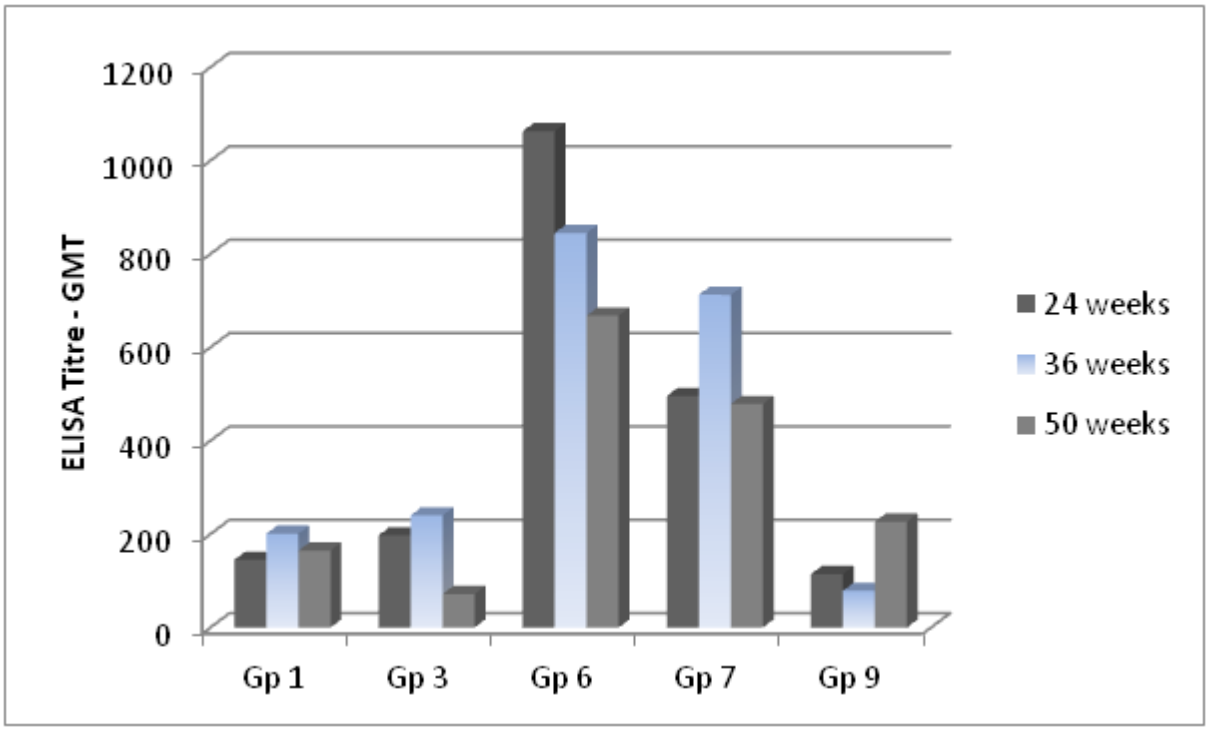

Figure 5-17 - Cage Reared – Egg Yolk IgY Antibody Levels

6 Discussion of Results

The major difficulty experienced within phase 1 was the low level of colonisation that was achievable with S. Typhimurium in the control birds. This difficulty increased with bird age. Better results were achieved with this serovar in Phase 2 but the difficulty remained at 22 weeks of age. Coincident with this we saw an increase in S. Typhimurium antibodies in control and oral vaccinated birds at around this age consistently in both phases (although this did not reach the positive threshold for the ELISA test). This may indicate a general non-specific increase in humoral antibody, perhaps related to the hen's physiological desire to produce antibody for inclusion into egg yolk (IgY, which is identical to Immunoglobulin G (IgG) as egg production begins (see Figures 5-9 and 5-13).

Vaccines have traditionally been developed to prevent disease. In most cases, vaccination will still allow infection with an organism but the disease will be prevented. The situation we face here is that the Salmonella serovars we are targeting do not cause a disease in chickens, even though the organism can effectively colonise (infect) the gastrointestinal tract. Asking a vaccine to totally prevent infection is an extremely demanding expectation. This, however, is the ultimate goal of a Salmonella control program in commercial chickens.

Older birds are also considerably more resistant to salmonellae than are young chicks and this is thought to be associated with the development of a microflora and considerably more salmonellae will adhere to caecal cells in two-day-old chicks than in chicks only a few days older (Gast, 2008). This resistance can develop within 36 hours of hatching (Barrow et al., 1999). Persistence of infection is also affected by bird age. Gast (2008) reports that the continued intestinal presence of S. Typhimurium was longer if given at one-day-old rather than at seven days. However, continued harbouring of the organism and intermittent faecal shedding has also been noted for up to one year and persistent carrier states have been described (Gast, 2008).
In addition, the number of salmonellae that may colonise the chicken's caeca is very low – often less than 100 cells per gram of caecum. To demonstrate a significant reduction in Salmonella numbers is thus also very demanding (Stern, 2008).

Shedding of salmonellae in chicken faeces can be intermittent and may continue for many months (Lister and Barrow, 2008).

For these reasons, the assessment of effective reduction in Salmonella colonisation in these studies was restricted to presence or absence in the caeca at three weeks following the challenge.

Deguchi et al. (2009) studied the protective effects of a multivalent inactivated Salmonella vaccine containing antigens of S. Typhimurium, S. Enteritidis and S. Infantis. They challenged vaccinated birds at five to six weeks of age with strains of S. Typhimurium, S. Enteritidis, S. Infantis and S. Heidelberg four weeks post- vaccination and evaluated caecal faecal shedding of these organisms for up to 14 days post- infection, showing significant reductions for all these serovars. Although faecal levels were lowered, the control birds also demonstrated a decline in faecal contamination levels. Faecal shedding of Salmonella does not necessarily reflect the continued presence of the organism in the caeca and eradication of the organism is much more difficult to achieve (Lister and Barrow, 2008). Salmonella can remain in the caeca for long periods of time and shedding and maintain an infection in a flock by cycling through susceptible birds (Lister and Barrow, 2008).

The development of humoral antibody to salmonellae does appear important in the maintenance of immunity against intestinal colonisation. The development of humoral antibody may also reflect the coincident development of cell mediated immunity which is thought to be more important with resistance to Salmonella infection. Killed vaccines however are not believed to provoke cell mediated responses and the obvious success of killed vaccines, both within this project and in use in the field globally, would suggest that humoral immunity is important or there is another mechanism whereby these vaccines work.

Hassan et al. (1991) demonstrated that experimental infection with S. Typhimurium resulted in production of antibody in intestinal contents and bile, as well as in serum. Hence we may also be achieving some intestinal antibody presence from the use of inactivated vaccine. It may be possible that some humoral antibody produced from the inactivated vaccine through bile excretion in a similar manner.

It would appear that to provide any long lasting resistance to Salmonella colonisation of the chicken gastrointestinal tract by the use of live or killed vaccines requires the development of sufficient serum antibody against the organisms. This does not necessarily imply that it is the serum antibody level that is protective, only that such serum levels reflect an associated level of protection by whatever method. In addition, the level of serum anti-S. Typhimurium antibody required to produce an associated significantly protective effect must be at least the positive threshold of the ELISA (x-OvO). This outcome is very similar to that determined in field studies in broiler breeders in Australia (A. Pavic, pers. comm.). Although the vaccine has difficulty in eliminating an existing infection, obtaining sufficiently high serum titres during rearing as a result of vaccination, enables the flock to rid itself of the salmonella present over a shorter period. This is probably due to the removal of susceptibility throughout most of the flock, removing the organism's ability to maintain its presence by continual cycling into new susceptible birds.

7 Conclusions

The oral or spray administration of the live *Ar0-A* deletion mutant attenuated S. Typhimurium vaccine used in this study provides only short-term protection against Salmonella colonisation of the chicken caecum. However, if this vaccine is administered by subcutaneous injection, protection is more significant and of considerable duration (up to 34 weeks).

The inactivated vaccine used provides long lasting protection if at least two vaccinations are given.

The most effective protection against homologous and heterologous serovars required delivery of the live attenuated vaccine by subcutaneous injection at six weeks followed by intramuscular injection of the killed vaccine at 12 weeks.

Of most importance is the production of significant humoral antibody associated with vaccination; a titre of at least the positive threshold of the ELISA (x-OvO) test (log_{10} 2.89 or 785 ELISA units), preferably higher, signifies protection.

The inactivated vaccine appears to provide protection against included serovars (homologous) but not against those not incorporated in the vaccine (heterologous). However, subcutaneous injection of the attenuated S. Typhimurium strain appeared to provide some cross-protection, at least against S. Infantis, but not S. Virchow.
The combined use of both the live and inactivated vaccine by injection was able to provide cross protection against a serovar that was not included in the killed vaccine components (S. Virchow).

If only considering S. Typhimurium, the Phase 2 study suggests that if the live vaccine is given orally after a single live vaccine injection, significant protection may be obtained. This approach may give some cross protection against S. Infantis but not against S. Virchow, though this aspect of the study needs to be repeated.

A difficulty with the use of the live vaccine by injection exists, as this is an off-label use, and at least at the dose rate used herein, may cause adverse reactions in some layer breeds. This needs further study and elucidation.

8 Implications

Salmonella is a major causative agent of food borne human disease and it is still thought that poultry products (eggs and meat) are the main sources of human food borne infections in Western countries (Van Immersel et al., 2005). Salmonella stands out as the most commonly reported microbiological agent responsible for food borne illness where eggs have been implicated as the cause. It has been estimated there are about 12,800 cases of egg-related salmonellosis per year in Australia, costing $44 million, and that the number of cases is rising (FSANZ, 2009).

Australian poultry farmers need to adopt the developing food safety programs being recommended by the chicken meat and egg industry governing bodies (Jackson and Underwood 2005. Vaccination may be a useful adjunct to these programs, particularly where one or more Salmonella serovars are endemic, and there is a desire to improve this situation; where significant human health issues have been identified or, where a producer wishes to enhance protection of the flock.

Salmonellosis is not a significant disease of chicken flocks in Australia and the use of a Salmonella vaccine program could not be expected to result in any benefits in terms of hen health or egg production. However the adoption of the use of vaccination to control Salmonella colonisation of Australian commercial layer flocks may provide a strategy that may allow reduction in the risk of human food borne salmonellosis from egg products. Where a producer has a significant level of Salmonella in flocks, this approach may assist in the reduction of that presence over a period of time.

The cost of the live vaccine and the inactivated vaccine is an important consideration. The cost of administration (about $0.10 per bird) needs to be added and it must be noted that two injections are required for best effect.

Given success in decreasing the presence of salmonellae in Australian flocks through the use of a vaccination strategy, it may be possible for the egg industry to develop marketing or quality programs similar to the British Lion brand program where vaccination against Salmonella, coupled with an accreditation program (not too dissimilar to the Egg Corp Assured program; AECL, 2009) provides a recognised high quality image for accredited products. If this was seen to be of benefit by the market, a premium price for accredited eggs could help offset the cost of the vaccination program.

9 Recommendations

This project has lead to a number of recommendations to the egg industry for vaccination strategies to reduce the Salmonella colonisation of Australian egg layer flocks.

Only programs involving injection of the vaccines studied will provide long lasting protection against Salmonella colonisation of the chicken intestinal tract.

9.1 Option 1

Use two doses of an inactivated vaccine containing representative serovars of those expected to be encountered in the production environment. Suggested ages of administration are six and 12 weeks. The vaccine must be administered twice to achieve protection. This approach could be chosen when the serovars included in the inactivated vaccine are desired to be controlled.

9.2 Option 2

Administer the live vaccine by subcutaneous injection at six weeks of age, followed by an inactivated vaccine aimed at the resident Salmonella serovars on the farm at 12 weeks of age. It is believed that this will provide the most efficacious result and would be the most likely approach to provide cross protection against a wider range of Salmonella serovars, but it must be noted that the injectible use of the live vaccine is off-label at present and the safety of this vaccine used in this manner must be more fully evaluated.

9.3 Option 3

Administer the live vaccine by subcutaneous injection at around six weeks and follow this with an oral dose (via drinking water) of the live vaccine at a later stage during rearing (16-18 weeks). This may not provide as wide a range of cross protection as Option 2. However, it would be considerably cheaper to implement.

It must be noted that the injectible use of the live vaccine is off-label at present and the safety of this vaccine used in this manner must be more fully evaluated.

10 References

AECL, 2009. The Egg Corp Assured (ECA) program. www.aecl.org/egg-corp-assured

Anon. (1999). Outbreak of Salmonella Serotype Muenchen Infections Associated with Unpasteurized Orange Juice. Centers for Disease Control and Prevention. Cited 05.07.2012
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4827a2.htm

Anon. (2009). Multistate Outbreak of Salmonella Infections Associated with Peanut Butter and Peanut Butter--Containing Products, Centers for Disease Control and Prevention. Cited 05.07.2012
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm58e0129a1.htm

Anon., (2012). Six Multistate Outbreaks of Human Salmonella Infections Linked to Small Turtles. Centers for Disease Control and Prevention. Cited 05.07.2012 http://www.cdc.gov/salmonella/small-turtles-03-12/index.html

Barrow, PA., Lovell, MA., Murphy, CK. and Page, K. (1999). Epidemiol. Infection, 123: 121-132.

Cox JM., Woolcock JB., and Sartor AL. (2002). The significance of Salmonella, particularly S. Infantis, to the Australian Egg Industry, RIRDC Project Report No. UQ-19E. Accessed 4 September 2011.

Deguchi K., Yokoyama, KE., Honda T. and Mizuno K. (2009). Avian Diseases 53:281–286

Engleberg NC., Dirita V., and Dermody TS. (2007). Schaechter's Mechanisms of Microbial Disease Fourth Edition, Chapter 17, 209.

FSANZ (2009). Public health and safety of eggs and egg products in Australia. Accessed 3 September 2010 www.foodstandards.gov.au/_srcfiles/Eggs_healthandsafety

Gast RK., Guraya R., Guard J and Holt PS. (2011), Avian Diseases 55, 243-247.

Gast, RK. (2008). Paratyphoid infections. In Diseases of Poultry, Saif, YM Ed. Blackwell Publishing, pp. 636-655.

Groves PJ., Cox J. and Pavic A. (2006). Poster- Proceedings of AAAP Meeting, Hawaii.

Groves, P.J. and Sharpe, S.M. (2012). Case report: adverse reactions following parenteral administration of live attenuated Salmonella vaccine in as train of layer chickens. Proceedings of the Australian Poultry Science Symposium, University of Sydney 23:232.

Hassan, JO., Mockett, D., Catty, D. and Barrow, PA. (1991). *Avian Diseases* 35: 809-819.

IMVS, (2009). Australian Salmonella Reference Centre, *Annual Report,* Adelaide, SA.

Jackson C and Underwood G. (2005). Bioproperties Development of salmonella vaccination strategies for the Australian poultry industry.

Lister, S.A. and Barrow, P. (2008). Enterobacteriaceae (Chapter 8). In Poultry Diseases 6th Ed. Pattison et al., Eds. Elsevier, Edinburgh. Pp. 110-145.

OzFoodNet Network Annual Report (2009). Commun Dis Intell 2010; 34(4):396–426. Accessed 3 September 2011.
Http://www.health.gov.au/internet/main/publishing.nsf/content/cda-cdi3404b.htm.

Pavic A, Groves PJ, and Cox JM. (2010). Avian Pathology 39(1) 31-39.

Pires, S.M., Vigre, H., Makela, P. and Hald, T. (2010). Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne Pathog. Dis.11: 11351-1361.

Sharpe SM. & Groves PG. (2011). Proceedings, Australian Poultry Science Symposium 22: 238

Snow, L.C., Davies, R.H., Christiansen, K.H., carrique-Mas, J.J., wales, A.D., O'Connor, J.L., Cook, A.J. and Evans, S.J. (2007). Survey of the prevalence of salmonella species on commercial laying farms in the United Kingdom. Veterinary Record 161:471-476.

Stern, N.J. (2008). Salmonella species and Campylobacter jejuni caecal colonization model in broilers. Poultry Science 87:2399-2403

Van Den Bosch, G. (2003). Vaccination versus treatment: How Europe is tackling the eradication of Salmonella. Asian Poultry Magazine – July

Van Immersel F, Methner U, Rychlik I, Nagy B, Velge P, Martin G, Foster N, Ducatelle R and Barrow PA (2005). Review Article – Epidemiology Infection. 1 – 20.

Xie, H., Rath, NC., Huff, GR., Huff, WE. and Balog, JM. (2000). Poultry Science 78: 33-40.

11 Appendix A

Table 11-1 Drag swab results from floor pens in rearing facility for Phase 2 (environmental samples)

11010648	Drag Swab	DOC	25/01/2011
Group	Pen	Age	Result
	1	0	NSD
	2	0	NSD
	3	0	NSD
	4	0	NSD
	5	0	NSD
	8	0	NSD
	25	0	NSD
	28	0	NSD
	29	0	NSD
	30	0	NSD
	31	0	NSD
	32	0	NSD

11010649	Drag Swab	DOC	25/01/2011
Group	Box	Age	Result
	1	0	NSD
	2	0	NSD
	3	0	NSD
	4	0	NSD
5	5	0	NSD

11020083	Drag Swab	DOC	2/02/2011
Group	Pen	Age	Result
	1	8	NSD
	2	8	NSD
	3	8	NSD
	4	8	NSD
	5	8	NSD
5	8	8	NSD
5	25	8	NSD
	28	8	NSD
	29	8	NSD
	30	8	NSD
	31	8	NSD
	32	8	NSD

11020268	Drag Swab	DOC	8/02/2011
Group	Pen	Age	Result
	1	14	NSD
	2	14	NSD
	3	14	NSD

	4	14	NSD
	5	14	NSD
5	8	14	NSD
5	25	14	NSD
	28	14	NSD
	29	14	NSD
	30	14	NSD
	31	14	NSD
	32	14	NSD

11020749	Drag Swab	DOC	22/02/2011		
Group	Pen	Age	Result	IMVS	Phage
	1	4-0	NSD		
	2	4-0	NSD		
	3	4-0	Gp B (Suspect Typh)	Typhimurium	108
	4	4-0	NSD		
	5	4-0	Gp B	Agona	
5	8	4-0	NSD		
5	25	4-0	NSD		
	28	4-0	Gp B (Suspect Typh)	Typhimurium	12
	29	4-0	NSD		
	30	4-0	NSD		
	31	4-0	NSD		
	32	4-0	NSD		

11030053	Drag Swab	DOC	1/03/2011		
Group	Pen	Age	Result	IMVS	Phage
	1	5-0	NSD		
	2	5-0	NSD		
	3	5-0	Gp B (Suspect Typh)	Typhimurium	108
	4	5-0	NSD		
	5	5-0	NSD		
5	8	5-0	NSD		
5	25	5-0	NSD		
	28	5-0	Gp B (Suspect Typh)	Typhimurium	108
	29	5-0	NSD		
	30	5-0	NSD		
	31	5-0	NSD		
	32	5-0	NSD		

11030051	Drag Swab	DOC	1/03/2011		
DS Feed Bag	3	5-0	NSD		
DS Feed Bag	8	5-0	NSD		
DS Feed Bag	28	5-0	NSD		
BPW 519	Control		NSD		

11030339	Drag Swab	DOC	7/03/2011		
Group	Pen	Age	Result	IMVS	Phage
	1	6-0	NSD		
	2	6-0	NSD		
	3	6-0	Gp B (Suspect Typh)	Typhimurium	12 & 108
	4	6-0	NSD		
	5	6-0	NSD		
5	8	6-0	NSD		
5	25	6-0	NSD		
	28	6-0	Gp B (Suspect Typh)	Typhimurium	108
	29	6-0	NSD		
	30	6-0	NSD		
	31	6-0	NSD		
	32	6-0	NSD		
Feed Bag A			NSD		
Feed Bag B			NSD		

11030735	Drag Swab	DOC	16/03/2011		
Group	Pen	Age	Result	IMVS	Phage
3	1	7-0	NSD		
3	2	7-0	NSD		
	3	7-0	No Sample		
	4	7-0	NSD		
	5	7-0	NSD		
5	8	7-0	NSD		
5	25	7-0	NSD		
	28	7-0	Gp B (Suspect Typh)	Typhimurium	108
2	29	7-0	NSD		
2	30	7-0	NSD		
1	31	7-0	NSD		
1	32	7-0	NSD		

11030749	Caeca	DOC	17/03/2011		
Bird (3365)	3	7-0	NSD		

11030816	Drag Swab	DOC	18/03/2011		
Group	Pen	Age	Result	IMVS	Phage
	3	7-0	Gp B (Suspect Typh)	Typhimurium	108

11031051	Drag Swab	DOC	23/03/2011		
Group	Pen	Age	Result		
3	1	8-0			
3	2	8-0			
	3	8-0	Gp B (Suspect Typh)	Typhimurium	108
	4	8-0			
	5	8-0			
5	8	8-0			
5	25	8-0			
	28	8-0			
	29	8-0			
	30	8-0			
1	31	8-0			
1	32	8-0			

11040462	Drag Swab	DOC	14/04/2011		
Group	Pen	Age	Result		
3	1	11-0	NSD		
3	2	11-0	NSD		
	3	11-0	NSD		
	4	11-0	NSD		
	5	11-0	NSD		
5	8	11-0	NSD		
5	25	11-0	NSD		
	28	11-0	NSD		
	29	11-0	NSD		
	30	11-0	NSD		
1	31	11-0	NSD		
1	32	11-0	NSD		

Note: Faecal samples collected from under challenged pens during Phase 2 studies.

Table 11- 2 Six week Challenge – Salmonella S. Typhimurium: Faecal samples collected from Trial Farm cages at 3, 6, 10, 13, 15, 18 days and caecum at 21 days post challenge.

Group	Treatment	Cages	3 days	6 days	10 days	13 days	15 days	18 days	21 days
1	Control	33	5/5	3/5	4/5	4/5	5/5	5/5	5/12
5	V0	34	5/5	5/5	4/5	3/5	4/5	5/5	7/12

Table 11-3 -10 week Challenge – S. Infantis: Faecal samples collected from Sydney University cages at 4, 7, 13, days and caecum at 20 days post challenge

Group	Treatment	Cages	4 days	7 days	13 days	20 days
1	Control	1, 7, 10	3/3	3/3	2/3	7/12
2	N6	4, 8, 9	3/3	3/3	2/3	3/12
3 & 4	VS6	2, 6, 12	3/3	2/3	1/3	7/12
5	Vsp0 VS6	3, 5, 11	3/3	2/3	1/3	2/12

Table 11- 4 - 10 week Challenge – S. Virchow: Faecal samples collected from Sydney University cages at 4, 7, 13, days and caecum at 20 days post challenge.

Group	Treatment	Cages	4 days	7 days	13 days	20 days
1	Control	16, 19, 21	3/3	2/3	1/3	6/12
2	N6	13, 18, 24	3/3	3/3	2/3	2/12
3 & 4	VS6	15, 17, 23	3/3	2/3	2/3	2/12
5	Vsp0 VS6	14, 20, 22	3/3	3/3	2/3	2/12

Table 11- 5 - 10 week Challenge – S. Typhimurium: Faecal samples collected from Sydney University cages at 4, 7, 13, days and caecum at 20 days post challenge.

Group	Treatment	Cages	4 days	7 days	13 days	20 days
1	Control	26, 31, 36	3/3	3/3	1/3	6/12
2	N6	27, 32, 34	3/3	3/3	2/3	3/12
3 & 4	VS6	25, 30, 33	3/3	3/3	2/3	3/12
5	Vsp0 VS6	28, 29, 35	3/3	3/3	2/3	2/12

Table 11- 6 - 16 week Challenge – S. Virchow: Faecal samples collected from Sydney University cages post challenge

Group	Treatment	Cages	7 days	20 days
1	Control	127, 129, 134	3/3	10/12
2	N6 N12	126, 131, 136	3/3	8/12
3 & 4	VS6 N12	128, 132, 133	2/3	3/12
5	Vsp0 VS6	125, 130, 135	3/3	4/12
Group	Treatment	Cages	7 days	20 days
1	Control	101, 106, 111	3/3	7/12
2	N6 N12	103, 105, 110	3/3	9/12
3 & 4	VS6 N12	104, 108, 109	3/3	6/12
5	Vsp0 VS6	102, 107, 112	3/3	8/12

Table 11- 7 - 16 week Challenge – S. Typhimurium: Faecal samples collected from Sydney University cages post challenge

Group	Treatment	Cages	7 days	20 days
1	Control	114, 120, 124	2/3	10/12
2	N6 N12	115, 118, 121	2/3	3/12
3 & 4	VS6 N12	113, 119, 123	2/3	3/12
5	Vsp0 VS6	116, 117, 122	1/3	4/12

Table 11- 8 - 22 week Challenge – S. Infantis: Caecum samples collected from Trial Farm pens 20 days post challenge

Group	Treatment	Pens	20 days
1	Control	14	11/12
2	N6 N12	11	3/14
3	VS6 N12	10	5/15
4	VS6 Vo18	13	1/12
5	Vsp0 VS6	12	4/13

Table 11- 9 - 22 week Challenge – S. Virchow: Caecum samples collected from Trial Farm pens 20 days post challenge

Group	Treatment	Pens	20 days
1	Control	24	10/12
2	N6 N12	20	12/14
3	VS6 N12	23	8/14
4	VS6 Vo18	22	4/12
5	Vsp0 VS6	21	7/13

Table 11- 10 - 22 week Challenge – S. Typhimurium: Caecum samples collected from Trial Farm pens 20 days post challenge

Group	Treatment	Pens	20 days
1	Control	16	3/13
2	N6 N12	17	2/14
3	VS6 N12	18	1/14
4	VS6 Vo18	19	1/12
5	Vsp0 VS6	15	0/13

12 Appendix B

12.1 Further Studies

12.1.1 Egg Yolk Antibody provided by vaccination

During Phase 1 we took the opportunity to collect eggs being produced by several of the vaccinated groups. Eggs were collected from groups 1, 3, 6, 7 and 9 at 24, 34, 40 and 50 weeks. Blood samples were also collected at various ages and all were assayed using the ELISA (x-OvO) kits (formerly a Guildhay test). The antibody levels in the egg yolks were compared.

12.1.2 Effects of breed on the possible adverse reaction seen to the live vaccine

The adverse reaction noted in Phase 2 is to be studied further to explore the possibility of breed differences in response to the live vaccine. Results of this procedure will fall beyond the final report date but will be reported separately when concluded.

Twenty-five one-day-old chicks of each of the following commercial chicken breeds will be obtained on the same day: Isa Brown layer, Hy Line layer, Hisex layer, Cobb 500 broiler, Ross 308 broiler. The birds by necessity will have to be obtained from different hatcheries relative to their ownership. The layer lines will be all females, the broiler lines will be as hatched.

Paper from chick boxes of each breed will be collected and cultured for Salmonella. Five randomly selected chicks from each breed will have blood samples collected and then be euthanized and their visceral organs cultured for Salmonella. These steps will be undertaken to determine if Salmonellae are brought in with any of the chicks and the blood test will be used to look for the presence of Salmonella Typhimurium antibody (maternal antibody).

Fifteen of each of the breeds will be placed in a cleaned pen at the Zootechny facility, Austral (hence we will have a total of 100 mixed breed birds in a single pen).
The birds will be identified to breed by a toe web split (as the layers and broiler breeds are not distinguishable from each other and toe marks are permanent). Birds will all receive the same feed (chick starter) from common feeders within the pens. Birds will receive numbered wing tags at four weeks of age when these can reliably be applied without a high chance of tag loss. At this time, five birds per breed will have blood samples collected for S. Typhimurium antibody.

At six weeks of age, vaccination with Vaxsafe ST is proposed as follows:

The birds of each breed will have the vaccine administered so that five each receive Vaxsafe ST at 108 by subcutaneous injection, five each receive Vaxsafe® ST at 106 by subcutaneous injection and five each receive Vaxsafe® ST at 108 by intramuscular injection. The remaining birds will receive a dose of sterile PBS as a sham inoculation and serve as controls. The birds will be observed closely for two hours. Rectal temperatures will be recorded on each after this time. Three birds will have S. Typhimurium lipopolysaccharide (LPS) administered at a similar dose to that expected from the vaccine

at 108 cfu/bird. All birds shall be closely observed for the next four hours and any reactions recorded. Birds will be bled at 12 hours post inoculation and plasma assayed for phosphorus, cholesterol, total protein, liver enzymes and glucose, (as suggested by Xie et al. (2000) in an experimental study of LPS (endotoxin) inoculation of chickens. Also white blood cells will be enumerated and a differential white blood cell (WBC) count performed. Plasma may also be assayed for Interleukin-6 (IL-6) which is an established estimator of acute phase reactions in chickens (Xie et al., 2000).
At eight weeks of age, all birds will be blood sampled for S. Typhimurium antibody (vaccine response). All birds will be humanely euthanized following the last sampling point.

12.1.3 Respiratory routes of administration of the live vaccine

Following a finding in Phase 2 where some birds exhibited a serologically positive titre for S. Typhimurium antibody following coarse spray vaccination, an attempt will be made to examine whether respiratory tract application of the live vaccine may elicit an immunological response. This experiment will extend beyond the final report date for this project and hence will be reported separately when concluded.

Groups of 15 ISA BROWN layers will be used as follows:

1 NIL – control
2 Live vaccine by coarse spray (droplets > 100 microns)
3 Live vaccine by fine spray (smaller droplets using an atomiser)
4 Live vaccine by eye drop administration at 106 cfu per bird
5 Live vaccine by intra-tracheal instillation at 104 cfu/ bird
6 Live vaccine by sub-cutaneous injection at 104 cfu / bird

Vaccines will be delivered at one-day-old and birds observed for any reaction for several hours.

All birds will have blood samples collected at 3, 4 and 8 weeks of age, submitted for S. Typhimurium ELISA testing.

13 Plain English Compendium Summary

Project Title:	
• AECL Project No.:	1US091
• Researcher:	Dr Peter Groves
• Organisation:	The Poultry Research Foundation, The University of Sydney
• Phone:	02 4655 0689
• Fax:	02 4655 0693
• Email:	peter.groves@sydney.edu.au
• **Objectives**	To determine the capability to restrict Salmonella colonisation of and Salmonella shedding from layer chickens after differing vaccination regimes using live and inactivated Salmonella vaccines; and, to identify one or more efficacious and cost effective vaccination regime(s) to limit and achieve a reduction of Salmonella intestinal colonisation in commercial layer chickens in Australia.
• **Background**	*Salmonella* is a major causative agent of food borne human disease and poultry products (eggs and meat) are often incriminated as sources for human infection. *Salmonella* vaccines are becoming available in Australia for use in poultry and their value in decreasing the ability of Salmonella serovars in colonising the digestive tracts of layer hens needs to be evaluated.
• **Research**	**This project evaluated a number of different applications of both live and killed *Salmonella* vaccines in inhibiting caecal colonisation of layer strain chickens. The most promising regimes identified were further studied to confirm results and to look at cross protection against other *Salmonella* serovars.**
• **Outcomes**	Oral administration of the live vaccine was found to give only short term protection. The use of the live vaccine by subcutaneous injection and the use of the inactivated vaccine by intramuscular injection did provide good protection against caecal colonisation by S. Typhimurium and other serovars around the time of sexual maturity in the hens. This age is most important as infection occurring then will make transmission via the egg shell much more likely. The best long term protection and most cross-protective outcome resulted from the initial injection of the live vaccine followed by injection of the inactivated vaccine several weeks later.
• **Implications**	Vaccination may be a useful adjunct to the food safety program for layer farms where one or more Salmonella serovars have been found on farm and there is a desire to improve this situation.