Live and inactivated vaccine regimens against caecal Salmonella Typhimurium colonisation in laying hens

PJ Groves,⁎ SM Sharpe,⁎ WI Muir,⁎ A Pavic and JM Cox

Objective In Australia, Salmonella serovar Typhimurium (S. Typhimurium) is the predominant zoonotic serovar in humans and is frequently isolated from layer hens. Vaccination against this serovar has been previously shown to be effective in broilers and the aim of this current study was to assess and determine the best vaccination strategy (live or inactivated) to minimise caecal colonisation by S. Typhimurium.

Methods A long-term experiment (56 weeks) was conducted on iSABROWN pullets using a commercial live aroA deleted mutant S. Typhimurium vaccine and an autogenous inactivated multivalent Salmonella vaccine (containing serovars Typhimurium, Infantis, Montevideo and Zanzibar). These vaccines were administered PO or by SC or IM injection, either alone or in combination. Pullets were vaccinated throughout rearing (to 18 weeks of age) and sequentially bled for antibody titre levels. The birds, vaccinated and controls, were challenged orally with a field isolate of S. Typhimurium at different ages, held for 21 days post-challenge, then euthanased and their caeca cultured for the presence of Salmonella.

Results None of the oral live-vaccinated groups exhibited lasting protection. When administered twice, the inactivated vaccine gave significant protection at 17 weeks of age and the live vaccine given by SC injection given twice produced significant protection at 17, 25 and 34 weeks.

Conclusions Vaccination regimens that included parenteral administration of live or inactivated vaccines and thus achieved positive serum antibody levels were able to provide protection against challenge. Hence, vaccination may play a useful role in a management strategy for Salmonella carriage in layer flocks.

Keywords layer chickens; poultry; Salmonella Typhimurium; vaccines

Abbreviations CFU, colony-forming units; S. Enteritidis, Salmonella enterica serovar Enteritidis; S. Typhimurium, Salmonella enterica serovar Typhimurium

Salmonella is the most commonly reported microbial agent responsible for human foodborne illness where eggs have been implicated as the cause.¹ ² Food Standards Australia New Zealand estimates that over 12,000 cases of human salmonellosis occur per year in Australia that may be linked to eggs or egg products.³ The Australian poultry industry differs from most other countries in that Salmonella enterica serovar Enteritidis (S. Enteritidis) is not endemic in chicken breeding and egg-laying flocks.⁴ ⁵ Salmonella enterica serovar Typhimurium (S. Typhimurium) is the most frequent serovar isolated from Australian egg layer flocks (28.3% of salmonellae isolations from this source) and this situation has been relatively stable for many years.⁵ ⁶ It is also the most frequently detected serovar from human salmonellosis cases in Australia, responsible for 28% of cases in 2009.⁶ Other serovars found at high prevalence in layer flocks are Infantis (18.3% of isolations), Mbandaka (5.4%), Singapore (5.4%) and Kiambu (4.7%), but these each account for less than 5% of human salmonellosis cases in Australia.⁶

In Europe, vaccination against S. Enteritidis has been important in reducing the prevalence of that serovar within the poultry industry, with a consequent decline in human S. Enteritidis cases.⁷ ⁹ This has been followed by targeting of S. Typhimurium and other serovars through vaccination of layers and breeder chickens.¹⁰ In Australia, an inactivated autologous Salmonella vaccine (Intervet [now MSD Animal Health, NSW, Aust]) has been used with success by some poultry companies in decreasing the prevalence of undesirable Salmonella serovars in meat chicken breeder flocks.¹¹ The evaluation of an inactivated multivalent Salmonella vaccine has also been performed in Japan¹² and the USA.¹³ A live aroA deletion mutant S. Typhimurium vaccine (Vaxsafe ST®, Bioproperties Pty Ltd, VIC, Aust) has also been released for use in Australia.¹⁴ This live vaccine is registered for oral administration to birds at day-old or any other age, with potential to circumvent early colonisation, and studies have been undertaken to register the use of this vaccine by a parenteral route in Australia.¹⁵ Assessment of reduction in Salmonella colonisation and shedding from infected hens is problematic and varies across the literature.⁴ Faecal shedding of Salmonella does not necessarily reflect the continued presence of the organism in the caecum and cloacal swabbing is not regarded as a good indicator of infection.¹⁶ Other studies have demonstrated significant differences in caecal colonisation between vaccinated and control birds over short periods. One study evaluated an attenuated S. Typhimurium vaccine applied at hatch and 10 days and subsequently challenged at 5 weeks of age and showed lower caecal colonisation against controls only at 2–5 days post-challenge, but results were similar to the controls from 7 to 14 days.¹⁷ Another study demonstrated that caecal Salmonella content became low or non-detectable by 4 weeks post-challenge in unvaccinated birds.¹⁸

A review of Salmonella infection in laying hens noted that most studies conducted with Salmonella colonisation in chickens have been short term (2 weeks) and used single administration of very
high infective doses and that this may not reflect the situation for
the whole productive life of a commercial layer hen. It could be
argued that if a vaccine merely lowers organism numbers compared
with controls for a short period but natural decline in organism pres-
ence is essentially the same after approximately 2 weeks, the vaccine
has not really achieved a long-term improvement compared with no
treatment. However, a decline in the cumulative amount of salmo-
nellae shed into the environment during this 2–3 week period could
still have beneficial effects in terms of overall challenge levels experi-
enced by the flock.

In the field it is more likely that exposure to Salmonella serovars is at
a low level and moves gradually through the bird population. Some
birds will be initially colonised for short periods, but will serve as a
source of infection for other, as yet unexposed, birds in the flock. In
this way the infection is maintained for considerable periods on a
flock basis. Some published studies have investigated the duration of
immunity from vaccination against salmonellae in chickens, but very
few have worked with S. Typhimurium.

The objective of this study was to determine the capability to restrict
S. Typhimurium colonisation of the gastrointestinal tract of layer
chickens over their productive life span after differing vaccination
regimens using live and inactivated Salmonella vaccines.

Materials and methods

Animal ethics

The animal procedures used in this study were jointly supervised by
The University of Sydney Animal Ethics Committee (approval no.
N00/8–2009/2/5144) and Birling Animal Ethics Committee (approval no.
1038/12/10US), the latter supervising procedures within Zootechny Pty Ltd’s facilities. All procedures were carried out in accordance with the Animal Research Act of NSW (1985) and Reg-
ulations (2005) following the NHMRC Guidelines (2008) and NHMRC/ARC Code of Conduct (2007).

Layer stock

Commercial day-old brown-egg layer chicks (Rhode Island Red ×
Rhode Island White hybrid) were obtained from a commercial
hatchery (Baiada Poultry Pty Limited, NSW, Aust). Birds were sup-
plied already vaccinated against Marek’s disease, Newcastle disease
and infectious bronchitis from the hatchery and, during rearing, all
birds received vaccinations against fowl pox (at 2 weeks of age; Inter-
vet/MSD Animal Health, batch no. 3961–009), Newcastle disease
(4 weeks of age; Vaxsafe® ND, Bioproperties Pty Ltd, batch
NDV073371A), infectious bronchitis (4 weeks of age; Vaxsafe® IB
(I), Bioproperties Pty Ltd, batch no. IB1062831A) and infectious lar-
yngotracheitis (8 weeks of age; Pfizer SA2, batch no. 1570114A), fol-
lowing common commercial practices using vaccines commercially
available in Australia.

Salmonella vaccines

Two vaccines were used in this study. An Australian-developed live-
attenuated aroA deletion mutant S. Typhimurium vaccine (Vaxsafe®
ST; Strain STM-1, batch no. STM071421A, Bioproperties Pty Ltd)
and coded as ‘V’, and an autologous multivalent inactivated Salmo-
rella vaccine (Intervet/MSD Animal Health, batch no. 4078A-031),
coded ‘N’. The inactivated vaccine contained field isolates of serovar
S. Typhimurium DT12 (serogroup B1), Infantis (serogroup C1),
Montevideo (serogroup C1) and Zanzibar (serogroup E1) at 10⁸
colony-forming units (CFU) of each serovar per bird dose. This vac-
cine contains thiomersal and formalin and was used under Australian Pesticides and Veterinary Medicines Authority (APYMA) permit number 10434.

Challenge organisms

The objective in this study was to simulate field conditions as closely
as possible; therefore, a recent field isolate of S. Typhimurium was
used rather than a laboratory-manipulated strain. A challenge strain
of S. Typhimurium DT108 was selected from a low-passage culture of
a recent poultry field isolation and was stored in Cryovials (Pro-
Lab Diagnostic, Ontario, Canada; REFPL.170/M) at −80°C.

For each challenge, a bead from a Cryovial was incubated in 100 mL
of buffered peptone water (Oxoid ThermoFisher, CM509, Hamp-
shire, UK) to produce a seed culture. Purity of the culture was
checked on nutrient agar and identity was confirmed serologically
using antisera (Pro-Lab Diagnostic; refs TL6002 [O], TKL6001 [H],
RL6011-04 [B]). Isolated colonies were selected and suspended in
9 mL of 0.1% peptone water to give a 75% transmittance (1.0 McFar-
land), equating to 2 × 10⁸ CFU/mL (bioMérieux, Marcy l’Etoile,
France; 47100-00 DR 100 colorimeter). The target inoculum required
for challenge or recovery experiments was achieved through decimal
dilution of the initial suspension in accordance with AS5013.10
(2009) (equivalent to ISO6579:2002, MOD), and confirmed by
spread plate enumeration on chromID™ Salmonella agar
(bioMérieux® Australia Pty Ltd, QLD, Aust; ref. 04913).

Vaccine trials

Management and sampling of stock. The chicks were placed in
floor pens at a research facility (Zootechny Pty Ltd) at 70 birds per
pen and reared to 13 weeks of age. The facility consisted of a small
commercial-style chicken shed with an insulated roof and side cur-
tains, providing floor pens of 6.5 m², each fitted with a bell waterer
and two tube feeders. A gas-fired space heater (Hired Hand®) pro-
vided artificial heat during brooding.

Commercial bagged layer starter, grower and laying rations
(Barastoc Feeds, Ridley Agriproducts, VIC, Aust) were fed to all
birds throughout the experiment. These feeds contained neither anti-
biotics nor any products such as organic acids that may inhibit
Salmonella.

Day-old chick box paper, as well as subsequent drag swabs of all
floor pens collected at 2, 4, 6, 9 and 11 weeks of age, were cultured for
Salmonella. At 13 weeks of age, reared birds were transferred to
individual layer cages at the Poultry Research Foundation’s poultry
unit (The University of Sydney, Camden, NSW, Aust) and main-
tained under production conditions.

The birds underwent a number of vaccination regimens involving
the live and inactivated vaccines by various routes and at differing
times as described in Table 1.

Challenge model. The challenge model used has been previously
described. At various ages (4, 12, 17, 25, 34 and 56 weeks) between
Table 1. Vaccination protocols using live and inactivated *Salmonella* vaccines in layer hens

| Group code | Vaccination regimen |
|------------|---------------------|
| C          | No vaccination – control group |
| V0-V3      | Live* vaccine PO at day-old and 3 weeks |
| V0-V3-N12  | Live* vaccine PO at day-old and 3 weeks and inactivated* vaccine IM at 12 weeks |
| V0-V3-V6   | Live* vaccine PO at day-old, 3 and 6 weeks |
| V0-V3-V6-N12 | Live* vaccine PO at day-old, 3 and 6 weeks and inactivated* vaccine IM at 12 weeks |
| N6-N12     | Inactivated* vaccine IM at 6 and 12 weeks |
| V0-V3-N6-N12 | Live* vaccine PO at day-old and 3 weeks; inactivated* vaccine IM at 6 and 12 weeks |
| VS4-VS8    | Live vaccine by SC injection* at 4 and 8 weeks |
| V0-V4-V14  | Live* vaccine PO at day-old, 4 and 14 weeks |

*Bioproperties Vaxsafe® ST by oral gavage at 10⁶ CFU/bird. 
*Intervet (MSD) Inactivated *Salmonella* vaccine at 0.5 mL/bird by IM injection. 
*Bioproperties Vaxsafe® ST by SC injection at 10⁶ CFU/bird. CFU, colony-forming units.

8 and 12 birds per vaccination group were selected at random and removed to experimental pens or cages in a different location and challenged with an oral dose of a field isolate of *S* Typhimurium. At 56 weeks of age the regimens that had not shown significant protection against colonisation at 17, 25 or 34 weeks were not tested.

The challenge dose was selected in an attempt to provide a realistic reflection of possible exposure in the field, without resorting to unrealistic levels. Successful colonisation of the control birds proved to be inconsistent at various ages. Hence, the challenge dose was varied over time, with 10⁶ CFU per bird used at 4 weeks of age and 10⁸ CFU at 10 weeks of age and thereafter. At 21 days post-challenge, the birds were euthanased and their caeca aseptically collected and cultured for the presence of *Salmonella*.

At 4 weeks of age, 10 birds were selected and identified from each group and were maintained unchallenged. These individual birds were bled at 9, 12, 14, 23, 31, 39 and 51 weeks of age to assess serological antibody response to vaccination alone. The serum was examined for the presence of antibody against *S* Typhimurium using a commercial ELISA kit (catalogue no. V020, x-OvO Ltd, Dunfermline, UK) following the manufacturer’s instructions. A positive reaction was taken as a sample/positive absorbance ratio > 0.25 at 550 nm, which equates to an ELISA titre of > 785 units (log10 2.89) as recommended by the manufacturer.

Preparation of live vaccine. The live *S* Typhimurium vaccine (1000-dose vial containing 10¹⁰ CFU) initially was diluted in sterile phosphate-buffered saline to a working dose concentration of 10⁶ CFU/250 μL. This dosage was given either by oral gavage using a stepper pipette (Finnpipette®), catalogue no. 4540, Thermo Electron Corporation, Waltham, MA, USA) or by SC injection using individual 1-mL hypodermic syringes and a 1-cm 22-gauge needle. The live vaccine preparation on each occasion was enumerated by performing decimal dilutions to 10⁻⁸ and then cultured duplicates of each dilution onto chromID™ *Salmonella* agar to quantify the amount given, using the same methodology as for the challenge inoculum.

Vaccination regimens. There were nine vaccination regimens (including a non-vaccinated control), coded V or N denoting the vaccine used, followed by the age at administration, in weeks (Table 1). Vaccine dosages used were at current rates recommended by the manufacturers. It must be noted that the manufacturers of Vaxsafe® ST have since reduced their recommended dose rate to 10⁷ organisms per bird for oral inoculation.

Caecal culture and *Salmonella* detection

All microbiological testing, including the vaccine and challenge strain enumeration, was performed at a NATA accredited laboratory (Birling Avian Laboratories) in accordance with AS5013.10-2009. The caeca were initially emulsified 1 : 10 in buffered peptone water and then enriched and further cultured as described in the Standard. Isolates were presumptively confirmed using validated commercial chromogenic agar chromID™ *Salmonella* agar (bioMérieux Australia).

Typical presumptive *Salmonella* were confirmed serologically with poly-O and poly-H and anti-serogroup B antisera (Pro-Lab Diagnostic; refs TL6002, TKL6001 and RL601104) and the slide agglutination technique after subculture on a nutrient agar slope. The confirmed *Salmonella* isolates were forwarded to the Australian *Salmonella* Reference Laboratory for complete serological and phage typing.

Statistical analysis

The proportion of birds for which *S* Typhimurium was isolated from the caeca was compared between each vaccinated group and the unvaccinated control group using contingency table analysis (Chi-square or Fisher’s exact test if an expected cell value was < 5), performed using the StatCalc function of EpilInfo™ (CDC, 2000). The quantitative serology results using the *S* Typhimurium ELISA were analysed using ANOVA and means were separated by Tukey’s honestly significant difference (HSD) test using Statistica™ (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.

Results

No environmental salmonellae were cultured from the day-old chick box paper or drag swabs collected in the rearing pens holding the unchallenged birds for the duration of the experiment.

The data summarised in Table 2 show the numbers of birds positive for caecal *S* Typhimurium 21 days after each challenge age. At 4 weeks of age, only groups vaccinated prior to this age (V0-V3 and V0-V4) were compared with control birds. The low levels of control group colonisation (< 30%) made difficult the determination of...
The overall objective of any intervention method for S. Typhimurium control in commercial laying hens is to reduce the amount of the organism that may be passed on via the egg into the human food chain. Hence, such a program must aim to reduce the long-term carriage of salmonellae in the gastrointestinal tract of layer hens and minimise the opportunity for human pathogenic serovars colonising hens during the laying period.

A notable difficulty in the evaluation of this study was the low levels of caecal colonisation in the control groups. The only ages at which caecal colonisation of control groups exceeded 50% were 17, 25 and 34 weeks, with the low colonisation levels in controls demonstrating statistically significant protection is impossible, even with zero colonisation of a vaccinated group. Achieving high levels of S. Typhimurium caecal colonisation in experimental control (unvaccinated)

---

**Table 2. Caecal colonisation of layer hens 21 days following challenge with Salmonella serovar Typhimurium**

| Vaccination protocol | No. of birds positive in caeca post-challenge at ages (no. challenged) |
|---------------------|---------------------------------------------------------------|
|                     | 4 weeks (n = 8) | 10 weeks (n = 8) | 17 weeks (n = 8) | 25 weeks (n = 10) | 34 weeks (n = 10) | 56 weeks (n = 12) |
| C                   | 1               | 2               | 5<sup>A</sup>    | 8               | 6<sup>A</sup>    | 4               |
| V0-V3               | 1               | 0               | 2<sup>AB</sup>   | 4               | 9<sup>A</sup>    | NT              |
| V0-V3-N12           | NT              | 0               | 2<sup>AB</sup>   | 7               | 9<sup>A</sup>    | NT              |
| V0-V3-V6            | NT              | 2               | 6<sup>A</sup>    | 3               | 5<sup>AB</sup>   | NT              |
| V0-V3-V6-N12        | NT              | 1               | 2<sup>AB</sup>   | 4               | 6<sup>A</sup>    | 4               |
| N6-N12              | NT              | 2               | 0<sup>B</sup>    | 5               | 6<sup>A</sup>    | 5               |
| V0-V3-N6-N12        | NT              | 1               | 2<sup>AB</sup>   | 7               | 5<sup>AB</sup>   | 1               |
| VS4-VS8             | NT              | 2               | 0<sup>B</sup>    | 3               | 1<sup>B</sup>    | 7               |
| V0-V4-V14           | 2               | 3               | 1<sup>AB</sup>   | 6               | 6<sup>A</sup>    | NT              |

**Statistical difference from control**

- **P > 0.05**
- **P < 0.05**
- **P < 0.05**
- **P = 0.07**
- **P > 0.05**

*<sup>A</sup>B: Means in the same column without common superscripts differ from the control group (P < 0.05).

NT, not tested.
chickens has been a common difficulty in many studies. For example, an early study with a live S. Typhimurium araA deletion mutant vaccine achieved only 20–50% of control birds when challenged at 11 days of age. A duration of immunity study using S. Typhimurium challenge, also using a live vaccine, recorded control positives of between 20% and 40% when challenged from 6 to 12 months of age, claiming long-lasting protection even though the later ages did not give statistically significant results. It appears that serovar Typhimurium infections in chickens are quite short term compared with the less invasive but more chicken-adapted serovar Enteritidis.

A review suggested that this may be caused by a more pronounced immune response to serovar Typhimurium, which allows it to be cleared more propitiously from birds. Attempts were made in the current study to improve the colonisation of control group birds with S. Typhimurium, firstly by increasing the challenge dose rate, then by increasing the number of birds per group and finally by incorporating mucin in the growth medium for the challenge cultures. An 80% positive colonisation in controls at 25 weeks of age was achieved, though at subsequent ages this rate

Figure 1. Mean log₁₀ Salmonella Typhimurium antibody ELISA titres in non-challenged birds by vaccination groups over time. (A) Groups that received only an oral dose of the live vaccine (Vaxsafe® ST: Strain STM-1, Bioproperties Pty Ltd, VIC, Aust; live-attenuated araA deletion mutant of S. Typhimurium). (B) Groups that received the inactivated vaccine (containing field isolates of serovar S. Typhimurium, Infantis, Montevideo and Zanzibar at 10⁸ CFU of each serovar per bird dose) by IM injection only at 12 weeks of age. (C) Groups that received the inactivated vaccine at 6 and 12 weeks of age or live vaccine by SC injection at 4 and 8 weeks of age. All charts show unvaccinated birds as a reference point and the x-OvO ELISA positive cut-off point (log₁₀ 2.89). Bars show standard error. CFU, colony-forming units.
declined sharply. It was more likely that this higher control colonisation at 25 weeks was related to the reduction in cell-mediated immunity in hens around point of lay, which has been shown to allow a resurgence of previously suppressed Salmonella infection, rather than to the changes in the challenge methodology. This possible mechanism and its effect with serovar Typhimurium needs to be studied more closely in chickens.

Of the vaccine regimens tested in this study, only dual injection of the inactivated vaccine IM (N6-N12), or the live vaccine SC (VS4-VS8), delivered significantly lowered S. Typhimurium caecal colonisation rates when challenged at 17 weeks. At 34 weeks, only the VS4-VS8 treatment significantly reduced colonisation, and all vaccination regimens failed to yield a significant protection at 25 weeks (P = 0.07 for V0-V3-V6 and VS4-VS8 compared with control group). The administration of the live vaccine by the oral route at various ages and numbers of repetitions failed to provide consistent or persistent protection against caecal colonisation. Administration of the live vaccine by the oral route prior to the use of inactivated vaccine did not improve protection against colonisation as compared with the inactivated vaccine used alone. These findings are consistent with another study using a live S. Typhimurium araA deletion mutant vaccine in broiler breeders where oral administration provided only short-term protection but subsequent injection of an inactivated trivalent vaccine produced a reduction in caecal colonisation at 22 weeks. A field study comparing a vaccination strategy using a combination of multiple oral live vaccinations followed by a killed bacterin of mixed Salmonella serovars to no vaccination in broiler breeders demonstrated lower caecal presence of Salmonella in the vaccinated birds, but this did not allow separation of effects from the individual vaccine types.

There was no detectable seroconversion seen with the live vaccine given orally in this study. It is important to note that this outcome may only be concluded for the particular vaccine used in this study and may not be the same for other live Salmonella vaccines. Live, orally administered Salmonella vaccines have been shown to elicit cell-mediated immunity and their protective ability to be mainly reliant on this component of the immune system. Serum antibody levels to S. Typhimurium in unchallenged, oral live-vaccinated chickens were no different to those of unvaccinated control chickens between 14 and 23 weeks of age (Figure 1A). The mean titre for these groups (C, V0-V3, V0-V3-V6 and V0-V4-V14) did not reach the positive threshold for the x-OvO S. Typhimurium ELISA test.

In contrast to our findings, an earlier study using the progenitor to the live vaccine studied here reported significant humoral responses following oral administration at 10^6 CFU per bird at 21 and 28 days post vaccination. That study used a different breed of bird and a custom-made ELISA, but the difference in the serology results with the current study is stark. This may indicate that the currently studied live vaccine lacked recognition by the host to the same extent as the earlier version. Those authors concluded that the vaccine organism was able to colonise the gut for 14–21 days before being eliminated and that this colonisation was necessary for contact with the host tissues long enough to establish a strong immune response. This response was not demonstrable in the current study despite multiple oral exposures at a high dose rate (10^6 CFU/bird).

Improvement in the control of Salmonella in commercial layer flocks to reduce the risk of foodborne illness requires a program to substantially decrease intestinal colonisation of hens just prior to and throughout their egg production lifetime. Hence, a vaccine must provide enduring protection at least until after the onset of sexual maturity, a physiological age at which cell-mediated immunity may be compromised. The difficulty in producing an experimental challenge colonisation of adult hens, as seen here and in other studies that have evaluated long-term protection, suggests that caecal colonisation by serovar Typhimurium in older hens may be naturally limited. It would appear that protection in the peri-maturity period, with its associated relaxation of cell-mediated immunity, may be the most important point in time at which a vaccine may provide a reduction in colonisation of hens throughout their entire egg production period. Although cell-mediated immunity may be suppressed at this time, adequate protection at this age may require the pre-existence of an effective humoral antibody presence in the chicken. The live vaccine given orally did not provoke humoral antibody production and if the existing cell-mediated protection was compromised around sexual maturity, the comparative failure of protection from this type of vaccine between 17 and 34 weeks of age may be explained. This possible mechanism requires further study.

Protection over any extended time was achieved in this study only where the vaccine, either live or inactivated, was administered parenterally. The inactivated multivalent vaccine used in this study provided significant protection after two applications when birds were challenged at 17 weeks of age but not thereafter, as measured under this challenge system.

In conclusion, oral application of the live S. Typhimurium araA deletion mutant vaccine used in this study did not provide resistance to colonisation of the caeca with a wild-strain of S. Typhimurium. However, administration of this live vaccine by the SC route provided strong and longer lasting protection from caecal colonisation. This route of administration of this attenuated vaccine may provide a useful approach to improved control of Salmonella in layer hens.

Further studies are needed to evaluate the best and most cost-effective method of use of injectable vaccines, including the initial administration of the live vaccine SC followed by the inactivated vaccine IM some weeks later. The ability of these vaccines, alone and in combination, to provide cross protection against other S. enterica serovars also needs to be examined.

Acknowledgments

This study was funded by the Australian Egg Corporation Limited. Vaccines and some financial assistance were provided by Bioproper- ties Australia and Intervet-Schering-Plough Australia (now MSD Animal Health Australia).

We thank Gavin Bailey and Taha Harris (Birling Avian Laboratories, Bringley, NSW) for their technical microbiological assistance and Jadranka Velnic and Susan Ball (Zootechny Pty Ltd, NSW) and Joy Gill and Melinda Hayter (University of Sydney, Camden, NSW) for assistance with bird husbandry.
References

1. Risk assessments of Salmonella in eggs and broiler chickens. In: Microbiological risk assessment series. 2. World Health Organization/Food and Agriculture Organization of the United Nations. http://www.fao.org/docrep/fao/005/y4392e/y4392e00.pdf 2002. Accessed January 2009.

2. Food Standards Australia New Zealand. Public health and safety of eggs and egg products in Australia: explanatory summary of the risk assessment. FSANZ, 2009. http://www.foodstandards.gov.au/publications/Pages/publichealthandsafetystandards.aspx. Accessed March 2015.

3. Food Standards Australia New Zealand. Public health and safety of eggs and egg products in Australia. FSANZ, 2009. http://www.foodstandards.gov.au/foodstandards/primaryproductionprocessingstandardsaustralianonly/eggstandard/. Accessed December 2011.

4. Wales AD, Davies RH. A critical review of Salmonella typhimurium infection in laying hens. Avian Pathol 2011;40:429–436.

5. Murray CJ. Salmonella serovars and phage types in humans and animals in Australia 1987–1992. Aust Vet J 1994;71:78–81.

6. Davos D, editor. Annual Report of the Australian Salmonella Reference Centre. IMVS, Adelaide, 2009.

7. European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA J 2012;10:2597, doi:10.2903/j.efsa.2012.2597.

8. van den Bosch G. Vaccination versus treatment: how Europe is tackling the eradication of Salmonella. Asian Poultry July 2003:1–3.

9. Woodward MJ, Gettinby G, Breslin MF et al. The efficiency of Salenvac, a Salmonella enterica subsp. enterica serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. Avian Pathol 2002;31:383–392.

10. Clifton-Hadley F, Breslin M, Venable LM et al. A laboratory study of an inactivated bivalent iron restricted Salmonella enterica serovars Enteritidis and Typhimurium dual vaccine against Typhimurium challenge in chickens. Vet Microbiol 2002;89:167–179.

11. Pavic A, Groves PJ, Cox JM. Utilization of a novel autologous killed tri-valent (serogroups B [Typhimurium], C [Mbandaka] and Orion [E]) for Salmonella control in commercial poultry breeders. Avian Pathol 2010;39:31–39.

12. Deguchi K, Yokoyama K, Honda T et al. Efficacy of a novel trivalent inactivated vaccine against the shedding of Salmonella in a chicken challenge model. Avian Dis 2009;53:281–286.

13. Berghaus RD, Thayer SG, Maurer JJ et al. Effect of vaccinating breeder chickens with a killed Salmonella vaccine on Salmonella prevalences and loads in breeder and broiler chicken flocks. J Food Protect 2011;74:727–734.

14. Alderton MR, Fahey KJ, Coleo PJ. Humoral responses and salmonellosis protection in chickens given a vitamin-dependent Salmonella Typhimurium mutant. Avian Dis 1991;35:435–442.

15. Abs El-Osta Y, Mohotti S, Carter F et al. Preliminary analysis of the duration of protection of Vaxsafe® ST vaccine against Salmonella shedding in layers. Proc Aust Poultry Sci Symp 2015;26:163–166.

16. Barrow PA. ELISAs and the serological analysis of Salmonella infections in poultry: a review. Epidemiol Infect 1992;109:361–369.

17. Dueger EL, House JK, Heitoff DM et al. Salmonella DNA methylase mutants elicit protective immune responses to homologous and heterologous serovars in chickens. Infect Immun 2001;69:7950–7954.

18. Stern NJ. Salmonella species and Campylobacter jejuni caecal colonisation model in broilers. Poultry Sci 2008;87:2399–2403.

19. Lister SA, Barrow P. Enterobacteriaceae. In: Pattison P, McMullin P, Bradbury JM et al., editors. Poultry diseases, 6th edn. Elsevier, Edinburgh, 2008,111–125.

20. Hassan JO, Curtiss R III. Efficacy of a live avirulent Salmonella Typhimurium vaccine in preventing colonisation and invasion of laying hens by Salmonella Typhimurium and Salmonella Enteritidis. Avian Dis 1997;41:783–791.

21. Wigley P, Hulme SD, Powers C et al. Infection of the reproductive tract and eggs with Salmonella enterica serovar Pullorum in the chicken is associated with suppression of cellular immunity at sexual maturity. Infect Immun 2005;73:2986–2990.

22. Bailey JS, Rolon A, Hofacre CL et al. Resistance to challenge of breeders and their progeny with and without competitive exclusion treatment to Salmonella Enteritidis. Int J Poult Sci 2007;6:386–392.

23. Dorea FC, Cole DJ, Hofacre CL et al. Effect of Salmonella vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. Appl Environ Microbiol 2010;76:7820–7825.

24. Springer S, Lindner T, Ahrens M et al. A new live Salmonella Enteritidis vaccine for chicken: experimental evidence of its safety and efficacy. Berl Munch Tierarztl Wochenschr 2000;113:246–252.

25. Hassan JO, Porter SB, Curtiss R III. Effect of infectious dose on humoral immune responses and colonisation in chickens experimentally infected with Salmonella Typhimurium. Avian Dis 1993;37:19–26.

(Accepted for publication 27 January 2016)