DEVELOPMENT OF SALMONELLA VACCINATION STRATEGIES FOR THE AUSTRALIAN POULTRY INDUSTRY

Clive Jackson[1] and Greg Underwood[2]

[1]Biological Technology Transfer Pty Ltd, 2 Victory Avenue, Camden NSW 2570

[2]BIOPROPERTIES Pty Ltd, 36 Charter St., Ringwood VIC 3134

1 Introduction

Australian poultry producers are coming under increasing pressure from food authorities and retailers to reduce the level of *Salmonella* contamination of all poultry products. Through the maze of industry quality assurance (QA) standards and government food safety schemes, producers are being required to implement hazard reduction programs commencing at the farming level through to the retail sector. Specific control programs are being implemented, such as the Food Safety HACCP Program for the Chicken Meat Industry (Australian Chicken Meat Federation [ACMF] 2005) and Egg Corp Assured (ACP), a national egg quality assurance program (Australian Egg Corporation Limited [AECL] 2005) designed to help commercial egg producers develop an approved quality assurance program for their business.

Successful control of *Salmonella* infections on poultry farms is reliant on good farming and husbandry practices (including all the aspects covering feed, birds, management, cleaning and disinfection, control of rodents, etc) as well as the testing and removal of positive flocks from production (The EFSA Journal 2004). Vaccination may be an additional option to a control program depending on the aim of the control program (reduction or eradication), type of poultry, stage of production, true prevalence of *Salmonella*, serovars targeted, detection methods used and cost-benefit. Vaccination may be a way of increasing the resistance of birds against S*almonella* exposure and decreasing the rate of shedding.

This paper aims to review the current use of *Salmonella* vaccines in a number of countries, provide a background to the development of BIOPROPERTIES (BPL) experimental *Salmonella typhimurium* (STM) vaccine, to be called Vaxsafe® ST Vaccine (living), and suggest how the vaccine could be used within a *Salmonella* control program in Australia.

2 Use of Salmonella Vaccines Overseas

The use of *Salmonella* vaccines in the European Community was recently reviewed by a scientific panel (The EFSA Journal 2004). In the summary of that report, it was evident that Europe is in the process of setting targets for *Salmonella* sp. and member countries will have to develop national

programs, beyond those currently adopted, to meet the new targets. Within those programs, vaccination is a specific control method that can be adopted. Currently, member countries vary widely in the adoption of vaccination. Twelve of 17 countries have mandatory/recommended *Salmonella* vaccination. Five countries do not allow vaccination. At the breeder level, eight use both live and inactivated vaccines and four use inactivated vaccines only. In layers, five countries use live vaccines only, five use both live and inactivated vaccines and one uses inactivated vaccine only.

The poultry industry in Europe has the choice of at least, nine live and six inactivated *Salmonella* vaccines. One of the licensed vaccines is Poulvac® ST (Fort Dodge Animal Health [FDAH], USA) which is derived from the same pre-master seed as Salvax (precursor of Vaxsafe® ST [BPL]).
In the USA, inactivated *Salmonella* vaccines have been used to control *Salmonella enteritidis* (SE) infections since the late 1980s. However, egg producers have been reluctant to adopt vaccination due to the cost of inactivated vaccines and the tissue reactions at the injection site (Kreager 1998). The more recent availability of live vaccines has resulted in wider adoption of vaccination to reduce both faecal and egg contamination with *Salmonella* sp.. Megan Health obtained a USDA license for a live vaccine in 1998, and in 2003, that company obtained a second license for a similar vaccine for layer pullets. Poulvac® ST (a product derived from the same master seed as Salvax (to be changed to Vaxsafe® ST) was licensed in the USA in 2000. In the 4 years since registration, sales have increased significantly whereby now the product has obtained a large share of the live *Salmonella* vaccine market, especially through the preferential use of the product in commercial broiler chickens (K. Cookson Pers. Comm.). This product can provide cross-protection against a number of *Salmonella* serovars, including *S. kentucky* (serogroup C), SE (serogroup D) as well as *S. heidelberg* and STM (serogroup B). (Cookson and Fan 2002, 2004; K Cookson Pers. Comm.)

In New Zealand, *Salmonella* control is required under the Poultry Industry Agreed Standards and Codes of Practice (PIANZ). The use of a live vaccine Megan® Vac 1 (Megan Health Inc, USA) is permitted and widely used in breeding flocks to aid in the reduction of *Salmonella* infection.

3 Advantages and Disadvantages of Vaccination

3.1 Advantages

- Vaccination can decrease the public health risk by reducing the colonisation of reproductive tissues as well as reducing faecal shedding. This results in lower levels of Salmonella in eggs and poultry meat.
- Vaccination can reduce the level of environmental contamination with wild-type Salmonella strains.

- There is no evidence of environmental contamination with live or inactivated vaccine strains.
- Live vaccines can be distinguished from wild-type strains by their growth on selective media.
- Parentally administered inactivated Salmonella vaccines will not interfere with the simultaneous application of competitive exclusion (CE) cultures or antimicrobials.

3.2 Disadvantages

- Additional cost, especially if inactivated vaccine administration requires additional handling of birds.
- Inactivated vaccines are usually targeted against a limited range of Salmonella serovars.
- Gene exchange between live vaccines and wild-type strains is theoretically possible.
- Parental administration by injection as well as the handling of poultry may be stressful and injection may be painful.
- Live vaccines could contaminate the end product if applied close to the time of slaughter or egg collection.
- CE treatment before the administration of live Salmonella vaccines will interfere with the live vaccination.
- Antibody responses to vaccination can cause confusion when testing for other Salmonella in accreditation and eradication programs (eg SE and Pullorum disease).
- Live vaccine efficacy can be interfered with by the prior administration of antibiotics or coccidiostats that have antimicrobial activity.
- Probiotics that block adhesion or have anti-Salmonella activity may interfere with live vaccination.
- Vaccines alone cannot guarantee freedom from Salmonella.

4 *Salmonella* vaccination in Australia – past, present and future

4.1 Past

Australian poultry processors and egg producers recognised the importance of *Salmonella* control over 30 years ago and instigated programs to reduce contamination of poultry products (Jackson, *et al.,* 1971). Huge efforts have continued to be made as chicken has become a principal food item in Australian households. Despite these efforts *Salmonella* contamination of poultry products has continued at a low level. Further reduction in contamination of poultry products continues to be the objective of the poultry industry.

Effective *Salmonella* control requires a multi-pronged programme involving biosecurity, supply of clean genetic stock, rodent control and feed / water treatment. Vaccination may be an additional option depending on the aim of the control program. *Salmonella* vaccination is most useful where the primary objective is *Salmonella* control (reducing the risk of *Salmonella* entry to a flock, or if entry occurs, limitation of shedding and therefore, contamination of poultry products). Vaccination programs are less useful if total eradication is the primary objective, however the present structure of the Australian layer and meat industries are arguably unsuited to elimination of all *Salmonellae*, and therefore control strategies are the primary local objective.

The need for *Salmonella* vaccines to assist in *Salmonella* control programs was recognised in the late 1980s, and a program to develop a live attenuated vaccine was initiated by Dr Peter Coloe at RMIT University with BPL (Alderton, *et al.,* 1991). That research culminated in the development of the STM-1 strain (an *aro*A deletion mutant) of *S. typhimurium* that formed the master seed for BPL's live Salvax *Salmonella* Vaccine, manufactured by Cyanamid Websters, Castle Hill, NSW from about 1994. The seed was also supplied to FDAH, USA for the manufacture of Poulvac® ST, currently licensed in the USA, Czech Republic and Slovakia. A number of batches of Salvax *Salmonella* Vaccine were used by Australian poultry companies to aid in *Salmonella* control during the mid-1990s. However, the closure of Cyanamid-Websters and changed NRA licensing requirements resulted in BPL requesting suspension of registration until new manufacturing facilities could be established. Accordingly, BPL has now established new manufacturing facilities at Glenorie and plans to seek re-registration of the product in the new premises and under the new name of Vaxsafe® ST Vaccine (living).

4.2 Present

Recently, the use of experimental inactivated autogenous non-SE *Salmonella* vaccines in Australian meat breeding flocks has been reported (Groves and Pavic 2005). This approach followed the successful reduction of SE infection in layer flocks in Europe using inactivated SE and ST vaccines. The authors reported a significant reduction in the colonisation of vaccinated breeding birds but could not demonstrate a significant protection in progeny challenged with virulent STM.

As mentioned above, there are no live or inactivated vaccines currently registered in Australia. However, permits may be obtained from the APVMA to conduct field trials with the experimental vaccine Vaxsafe® ST Vaccine (living) and/or inactivated autogenous

Salmonella vaccines. Possibly, for this reason, the current Australian industry food safety QA programs do not specifically mention the use of *Salmonella* vaccines. However, a RIRDC report on *Salmonella enteritidis* surveillance and response options for the Australian Egg Industry (Sergeant, *et al.,* 2002) recommended the use of vaccination in response to an outbreak of SE. It further recommended that AEIA (now AECL) should investigate the supply of vaccine and permits for the emergency supply of vaccine.

4.3 Future

It should be anticipated that vaccine companies will progress their registration applications over the next year and both live and inactivated vaccines against *Salmonella* sp will become available. It could also be anticipated that the advantages to be gained from the new vaccines could follow the claims made for comparable product manufactured overseas. Hence, the following programs may well be recommended:

Live vaccination

- A single dose should be applied at day-old by coarse spray and again at 14 days by drinking water to protect broilers.
- In addition to vaccination at one and 14 days of age, long-lived birds may be re-vaccinated at 10-12 weeks of age as part of a priming strategy before administration of an inactivated vaccine.

Inactivated vaccination

- Inject at 10-12 weeks and/or 2 weeks before lay to protect layers or breeders to reduce wild-type *Salmonella* infection and to reduce the incidence of horizontal and vertical transmission.
- Inactivated vaccines stimulate higher levels of serum antibody (compared to live vaccines) in parents, thus maternal antibody is transferred to the progeny which can protect from clinical disease / reduce infection rate of young flocks, particularly up to three weeks of age.

Combined live and inactivated vaccination program

The primary outcome following implementation of a vaccination programme should be a significant reduction in colonisation of paratyphoid *Salmonella* sp., including STM, other group B serovars, as well as serovar C and D *Salmonellae* (including SE). A program involving the use of both types of vaccines offers the advantages of stimulation of both the cellular and humoral arms of the immune system. It is generally considered that cellular or

local immunity at the epithelial surface of the intestine is stimulated by live vaccines, which is more effective in preventing colonisation through increasing the infectious dose threshold for the wild-type strains to become established. Inactivated vaccines predominantly stimulate humoral immunity, which is most beneficial in stopping the development of a bacteriaemia and therefore clinical disease and colonisation of the oviduct. Antibody is therefore more important for the control of SE, and other paratyphoid strains, including STM, that are transmitted vertically and spread horizontally in chicks under 2 weeks of age. A weakness of live vaccines however is that they generally induce relatively short periods of immunity (6-8 weeks), compared to inactivated vaccines that stimulate high levels of antibody, and in turn the transfer of protective maternal antibody to progeny, throughout the life of the flock. However, maternal protection may only last for the first 3 weeks of life, thus providing little protection from subsequent horizontal transmission such as during thinning in the broiler industry around 35 days of age, which is one of the highest risk critical control points of *Salmonella* infection in broiler flocks.

A suggested vaccination program for layers / breeders is shown in Figure 1 below and can be summarised as follows:

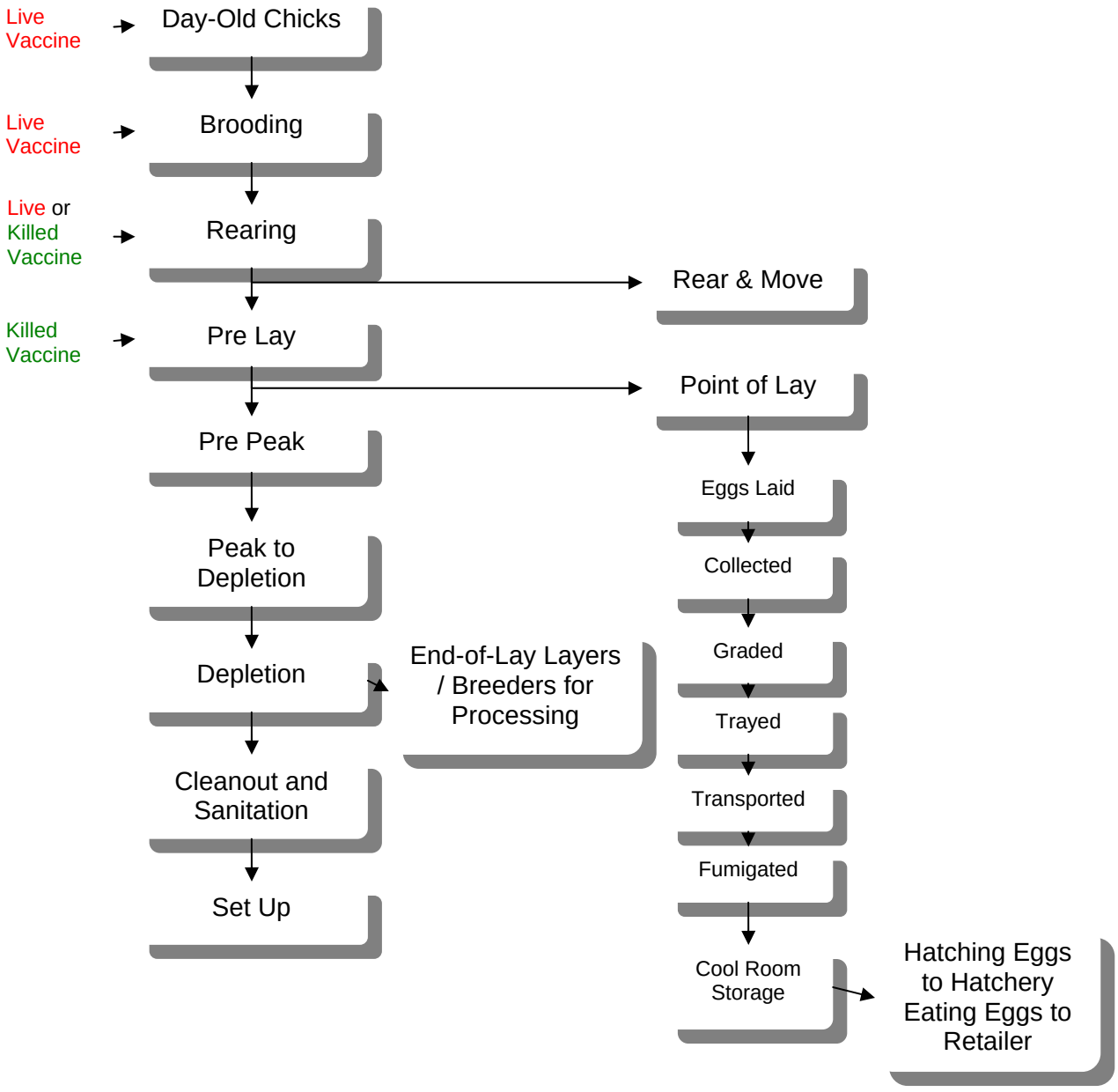
Program	1st vaccination	2nd vaccination	3rd vaccination	4th vaccination
A	Day-old (live) spray in hatchery	2 weeks (live) in drinking water	10-12 weeks (live) in drinking water	16 weeks* (inactivated) by injection
B	Day-old (live) spray in hatchery	2 weeks (live) in drinking water	10-12 weeks (inactivated) by injection	16 weeks* (inactivated) by injection

* Administer 2 weeks before laying commences.

Again, vaccination should be viewed as only one process in a multi-pronged *Salmonella* control program. Successful control of *Salmonella* infections on poultry farms is reliant upon good farming and husbandry practices including rodent and insect control programmes, feed and water treatment programmes, and a high level of farm biosecurity. Vertical / paravertical transmission from breeder flock to progeny is perhaps one of the greatest critical control points for entry of paratyphoid infections. It is essential that breeder flocks engage the highest control programme practices (including vaccination), together with a thorough environmental monitoring programme for early detection of infection, which will allow control strategies to be implemented to reduce the vertical shedding rate and in turn the infection level of progeny flocks. Even if live vaccination programmes are employed in day-old chicks, the vaccination process can not be expected to work without sufficient interval between

vaccination and challenge to allow immunity to develop. Thus the long-term control of *Salmonella* in the Australian poultry industry will rely heavily upon the ability of the breeder companies to control infection in the higher tiers of the breeding pyramid.

Figure 1. Process Flow - Layer / Breeder Life Cycle



5 Conclusions

Due to increasing pressure from food authorities and retailers to reduce the level of *Salmonella* in food products, Australian poultry farmers will need to adopt the developing food safety programs being recommended by the chicken meat and egg industry governing bodies, ACMF and AECL, respectively. *Salmonella* reduction requires a multi-pronged approach targeting all possible sources of *Salmonella* exposure. Overseas, many countries have included *Salmonella* vaccination with both live and inactivated vaccines as an additional method of control. Whilst there are a number of disadvantages of adopting *Salmonella* vaccination, it has clearly been established overseas that *Salmonella* vaccines can assist in increasing the resistance of birds against *Salmonella* exposure, decrease the rate of shedding, and significantly reduce the contamination of eggs / egg products and chicken meat. This, in turn, will maintain or improve consumer confidence in poultry products.

6 References

Alderton M R, Fahey K J and P J Coloe (1991) Humoral response and *Salmonella* protection in chickens given a vitamin-dependent *Salmonella typhimurium* mutant. *Avian Diseases*. 35:435-442.

ACMF (2005) Food safety HACCP programme for the chicken meat industry, 30th May 2005. Draft version distributed by ACMF on 1 June 2005, pp 1-84.

AECL (2005) Egg Corp Assured, The National Egg Quality Assurance Program – egg business guidelines. Bulletin pp1-2.

Cookson K C and H Fan (2002) A comparison of 3 live *Salmonella* vaccines against group C and D *Salmonella* challenge. Proc. 51st West. Poult. Dis. Conf. Puerto Vallarta, Mexico, pp 12-13.

Cookson K C and H Fan (2004) Efficacy of a live *Salmonella typhimurium* vaccine given by different routes and the influence of same-day antibiotic administration. Proc.53rd West. Poult. Dis. Conf., Sacramento, CA. pp 49-50.

Groves P and T Pavic (2005) Experiences with an inactivated *Salmonella* vaccine. Proc. 9-10 February Meeting AVPA, pp19-20.

Jackson C A W, Lindsay M L and F Sheil (1971) A study of the epizootiology and control *of S. typhimurium* infection in a commercial poultry organisation. *Aust. Vet. J.* 47: 201-206.

Kreager K (1998) Egg industry initiatives to control *Salmonella*. Proc International Symposium on food-borne *Salmonella* in poultry. July 25-26, Baltimore, Maryland, USA, pp 75-80.

Sergeant E S G, Grimes T G, Jackson C A W, Baldock F C and I F Whan (2002*) Salmonella enteritidis* surveillance and response options for the Australian Egg Industry. RIRDC Project No. AUV-A1, May 2002, pp 1-59.

The EFSA Journal (2004) The use of vaccines for the control of *Salmonella* in poultry. 114: 1-74.