



Salmonella Enteritidis Vaccine Feasibility

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A report for Australian Eggs

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Foreword

“Salmonella is the principal pathogen of concern associated with eggs and egg products.”¹.

Australia has 4.1 million cases of gastroenteritis per year, 55,000 of which are due to *Salmonella*².

The cost of food borne illness to society is about \$1.25 billion.

Cox and Pavic³ emphasised that the control of enteric pathogens transmissible to humans from poultry sources require the insertion of hurdles for the bacteria which span the entire poultry production chain, from elite breeder flocks down to final product for consumption. These strategies include biosecurity, poultry feed sterility, water control, egg production hygiene, rodent control, use of probiotics, prebiotics and other feed additives and vaccination. The hurdle that is currently missing in Australian egg production is a specific vaccine against *Salmonella* Enteritidis (**SE**).

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Summary

The current Australian approach to managing farms with a positive SE detection is not aligned to best practice in other countries and has a devastating impact on infected farms, few of which are able to avoid farm shutdown and cessation of business.

Overseas, where SE has been a persistent problem for decades, the level of SE in industry has been substantially reduced. Vaccination specifically against SE has played a strong part in decreasing the problem overseas. There is documented evidence from UK that associates major improvements in SE incidence in poultry flocks and in human cases with the introduction of SE vaccination in egg layer flocks. SE vaccination in Europe and USA is now ubiquitous.

In Europe and USA, there is no mandatory depopulation enforced on farms detecting SE. The farmer can decide on how to manage an incident. Decontamination of the site following a positive flock requires only a reasonable standard cleanout involving a dry clean and disinfection using an approved spray, aerosol or fumigation. All rearing flocks are part of the routine monitoring process.

The farmer can choose to depopulate, or divert all eggs to pulping (pasteurization), or undertake an egg testing protocol to provide evidence that there is no egg transmission of SE. The latter involves culture of 4000 eggs over 8 weeks at 2-week intervals. This sampling regime would provide a 99% confidence of detecting a single positive egg if the infection level in the eggs was 1%. If no SE is demonstrable in this egg sample, the farm returns to normal supply of table eggs, under continued environmental testing.

Under the biosecurity order active in NSW, biosecurity, rodent control and egg hygiene practices have been upgraded and audited. But we lack a specific vaccine against SE. Our studies with the NSW SE strain have shown partial protection against colonisation with SE but not complete protection against infection of the reproductive tract provided by an enhanced program of vaccination with the existing registered ST vaccines. An Australian study with a combination of the live registered vaccine and an SE bacterin has shown promise for long term protection of the reproductive tract and hence, protection from internal infection of the egg. Autogenous SE bacterins are only allowable for use in operations where SE was previously isolated.

There is an opportunity to explore the development of minor use permit bacterins, based on our SE strain, perhaps with other serovars such as ST and SI added. With industry and veterinary support, this could be deployed rapidly by existing vaccine manufacturers and laboratories until such time as a fully registered SE vaccine could be achieved (possibly two years away). The use of specific SE vaccines in a flock of layers should remove the mandatory depopulation/ decontamination imposition and allow the opportunity for farms to show no presence of SE in eggs where it may have been detected in the environment.

The focus of this paper is the ability to prevent transmission of *Salmonella* Enteritidis to human consumers via the egg. This is the critical problem and the vital requirement. If this can be reliably prevented, the temporary detectable presence of the organism in the flock or its environment is of much less importance.

The prevalence and threat of *Salmonella* serovar Enteritidis in Australia and impact on industry sustainability

Salmonella serovar Enteritidis (SE) has been a predominant problem for the poultry industry, particularly egg production, for many years overseas and Australia enjoyed virtual freedom from this serovar in commercial operations until 2018, when a contamination of a farming and grading operation preceded the spread of SE through multiple farms in NSW and into Victoria and Queensland. The particular SE strain was unique, was not related to any overseas strain and seemed to be of Australian origin.

The regulatory response was to try to eradicate it, by depopulating the affected farms, destroying all eggs and requiring stringent measures to decontaminate and subsequently prove that the facility was free⁴. Very few farms were able to meet these requirements and most closed down or were diverted into other uses.

Despite this approach, this very same SE strain has continued to cause food poisoning incidents, many of which could not be traced to egg producers. In 2023 and 2024, individual farms were found to be contaminated with this SE strain.

The current regulatory approach to farms when SE has been detected is complete depopulation without compensation. The farm owner has to cover the entire costs of euthanasia of all birds on site, disposal of the carcasses, full decontamination of the farm and all the subsequent testing to prove that SE has been eradicated from this site.

With Avian Influenza virus, if you take the birds away the virus cannot survive long, so eradication of this is highly possible. But with a bacterium like SE that can survive in the environment and other vectors, eradication is very challenging. SE will survive and reside in rodents, insects, soil, water, dust and other sites. Eradication from a farm is almost impossible to guarantee. There are also consequences for other operations if they remain associated with previously infected premises and usually means facilities will no longer be usable (grading floors, other associated farms).

The costs here are incredibly high (hundreds of thousands of dollars at least), the outcome is extremely difficult to achieve and most often, the farmer gives up and goes out of business.

Currently, egg farms in NSW remain under a biosecurity order for SE. This mandates regular environmental sampling for the detection of SE (every 12-15 weeks on all egg producing operations). This is a major threat to egg industry sustainability and the secure supply of eggs to the community as the consequences of a positive detection of SE are devastating. For smaller operators or those with only one or two premises, the detection of SE means being put out of business. As SE does not generally cause symptoms in the birds, there is no indication or warning that a test may come back positive. Further, there are no real preventative procedures available to deal with a minor SE introduction. None of the protective measures in use (strict biosecurity, rodent control, feed additives, probiotics or the current registered ST vaccines provide complete protection). A specific SE vaccine would greatly enhance protection against this eventuality.

There are also indirect effects of the risk of an SE detection. These concerns are a disincentive to invest in egg producing facilities, as one simple detection will cause major losses. Operators with only one or two production sites, even if they are large ones, are at extreme risk of financial ruin. Many farmers are considering leaving the industry. Family farms, which have been the backbone of the

layer industry, may disappear, leaving only large, multi-site producers.

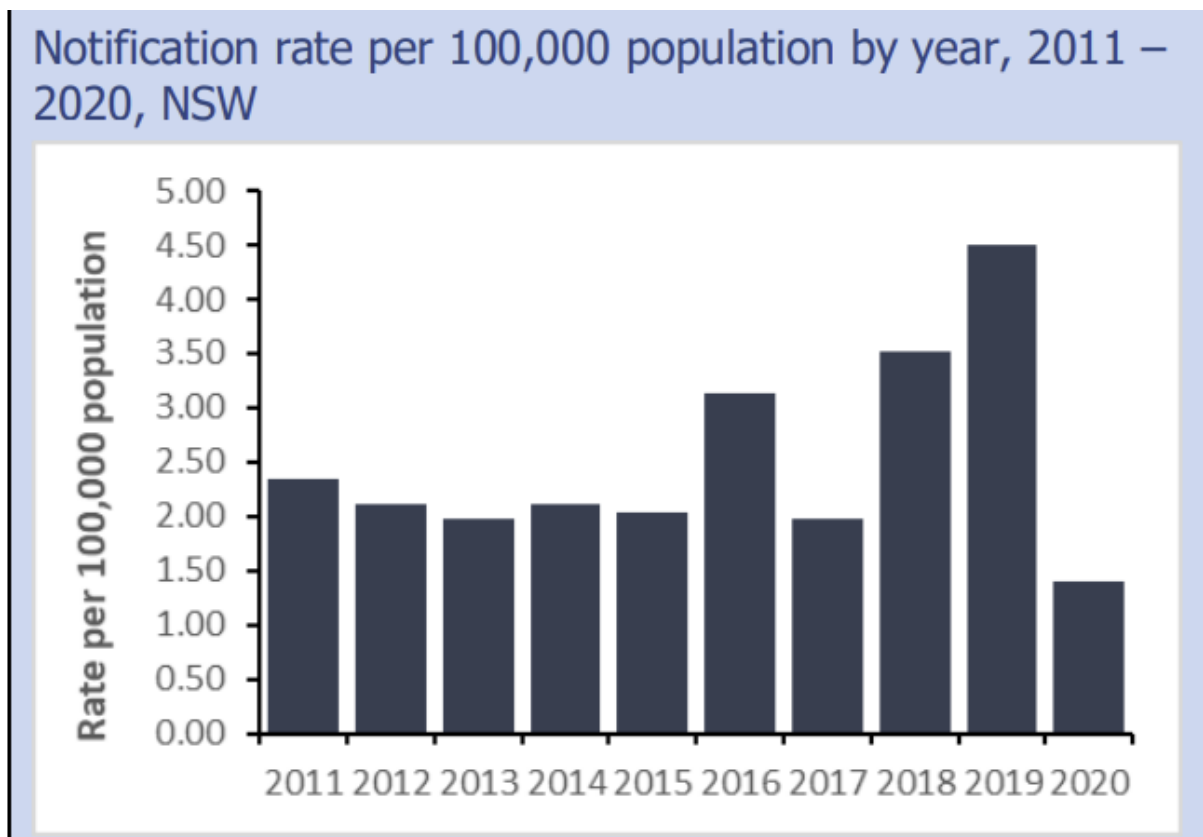
There are also flow-on consequences for bird health, welfare and food safety. Some farmers may be reluctant to allow any bacteriological diagnostic testing in the fear of accidentally finding SE (even though the risk of this is extremely low). Hence, diagnostic testing for other diseases, such as fowl cholera, spotty liver disease, colibacillosis and erysipelas is inhibited and the ability to perform antibiotic sensitivity testing prior to treatment is prevented. This last restriction may cause inappropriate and ineffective treatment of other conditions and contribute to antibiotic resistance development.

There is also reluctance to conduct any structured testing for sites of *Salmonella* (any serovar) presence or source of contamination, which limits further proactive *Salmonella* prevention strategies.

Some operations may also be tempted to not conduct routine SE monitoring with sufficient rigour.

All these consequences of the current approach to SE control establish barriers to effective veterinary service, adequate disease control, minimisation of antimicrobial resistance development and bird welfare. As such, this also represents increased risks to the egg consumer.

Despite the eradication attempts so far conducted, human occurrences of SE gastroenteritis continue, some of which are due to the peculiar SE strain found in NSW in 2018. The chart below shows the peak of SE human cases in 2018-2019 but the serovar retained prevalence in humans in 2020 and has continued in more recent years.



SE cases in humans in NSW: Source: OzFoodNet NSW Annual Report 2020 (www.health.nsw.gov.au)

Sporadic human cases with the NSW SE strain continue since 2020. Occasionally, this strain has been detected on farms (Windsor in 2023; Wallerawang in 2024). So, there must be an undetected reservoir of this strain somewhere (perhaps multiple sites) with access to the industry. As it stands,

the spread of the organism is unpredictable.

Following the 2018-19 outbreaks, NSW has a standing Biosecurity Order, requiring high attention to biosecurity, farm and egg hygiene, rodent control, feed and water quality and regular monitoring. These requirements are very similar to those adopted overseas. Overseas, industries adopted effective SE vaccination procedures as an integral part of *Salmonella* management strategies. But in Australia, we struggle on without an SE vaccine available. Vaccination is the missing barrier for SE protection in Australia.

Research combining and analysing studies of practices to reduce *Salmonella* in broiler chickens were conducted by Sarah Totton and others^{5,6} in Canada (2011 and 2012). The analyses compared published results looking at disinfection, vaccination, and feed and water additives on successful *Salmonella* control. She found that the strength of evidence for effects of feed and water additives and disinfection was low and only vaccination gave a significantly reliable outcome.

Successful preventative SE vaccination processes

Currently in Australia, the adopted preventative measures for protection against the incursion and establishment of SE into a poultry flock relies on farm biosecurity, hygiene and the hope that the use of a live *Salmonella* Typhimurium (ST) vaccine may provide cross-protection against SE. It is known that any cross-protection afforded by the available live ST vaccines in Australia is only partial (research studies from the University of Sydney). Our current practices reduce but can't completely eliminate the risk of SE introduction⁷.

Success in control of SE (and other *Salmonella*) has been reported overseas. Levels of human salmonellosis in the UK, particularly with SE, rose drastically during the early 1980's but showed a decline after a peak around 1997, associated with the introduction of inactivated vaccines against SE⁸. Wigley⁹ recently reflected on this in the UK, which was originally severely affected by the incursion of SE into its breeder and egg layer flocks in the late 1980's. A well-remembered statement by a health minister in 1988 started a series of "health scares" associated with poultry products. Eventually, legislation was introduced for hatchery cleanliness (as SE is transmitted in and on the egg) and the establishment of a **voluntary** scheme in the UK ("Lion Mark") which standardised SE surveillance testing, better biosecurity and "**crucially, vaccination**"⁹. This program successfully reduced SE presence in poultry flocks and there was a consequent decrease in human cases of food borne infection. This was subsequently adopted by the EU (legislated as the National Control Plans). These include vaccination as an integral part.

A report where vaccination against SE was not fully successful associated the poor vaccine performance with failure to control severe rodent infestations¹⁰.

A similar rise in human SE infections was seen in the USA¹¹ from 1985. In USA, live vaccines are considered to provide the best protection. New regulations¹² for *Salmonella* involving monitoring programs in meat chickens were in force by 2024. Vaccination was advised to assist reduce *Salmonella* shedding, noting the availability of both live and inactivated vaccines in USA – all live vaccines for broilers are ST mutants, but inactivated vaccines are designed for use in adult breeder hens to provide protection to the offspring, which is also the most appropriate approach for layers (i.e. protect the egg). It was stressed that vaccines are not a solution on their own.

Live SE vaccines may not achieve complete protection against intestinal colonization by wild SE strains but a study combining a competitive exclusion product and the live SE vaccine given at day old in chicks was able to eliminate liver colonization by the wild bacterium¹³.

Britain

Lane et al.¹⁴ described the changes in SE case incidence in the UK up to 2011. Following the incursion of SE into UK layer flocks, the situation was dire, and the level of human SE cases continued unchecked for several years. The figure below shows the rapid increase in SE cases in the late 1980's, an uncontrolled peak through till 1998 and a major decline subsequently.

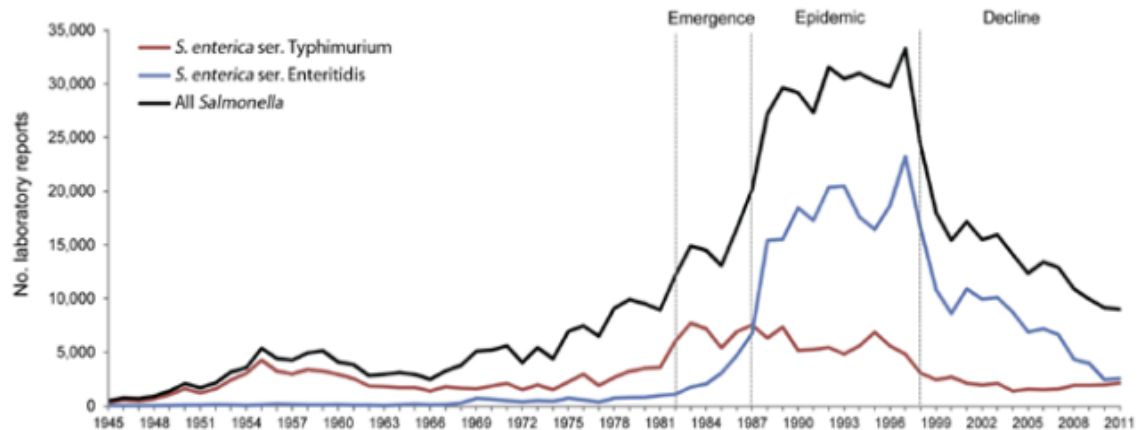


Figure 1. Laboratory reporting of *Salmonella enterica* infections in England and Wales, 1945–2011. Emergence stage, 1982–1987; epidemic stage, 1988–1998; decline stage, 1999–2011. Ser., serovar.

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Source: Lane et al., 2014

The second figure from this source (below) shows the major decrease in presence of detectable SE in all UK poultry flocks correlated with the introduction of an inactivated SE vaccination in poultry breeders but a decrease in human SE cases was not achieved until SE vaccination was introduced into egg layer flocks in 1997. The SE vaccine was not specific for SE DT4 (which was their major outbreak strain) and its use was voluntary, but well adopted, by industry.

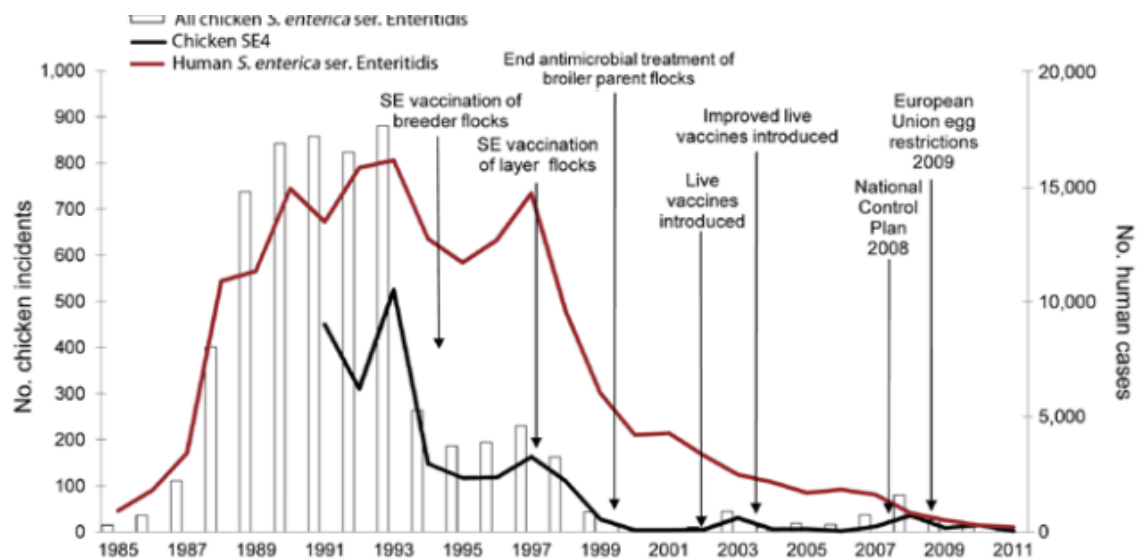


Figure 5. Trends in the reporting of incidents of *Salmonella enterica* in chickens in Great Britain versus laboratory reporting of human *S. enterica* serovar Enteritidis infection, England and Wales, 1985–2011. SE4, *S. enterica* ser. Enteritidis phage type 4.

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Source: Lane et al., 2014.

The reality of the association between the introduction of layer vaccination and the reduction in human cases has been well established. For example, to quote Gibbons¹⁵ from a 2012 website: “The research, conducted by the Liverpool Institute of Infection and Global Health, revealed the “remarkable decline” in non-typhoidal salmonellosis in the UK since the late 1990s. This coincided with the introduction of voluntary vaccination programmes in broiler-breeder and laying flocks, such as the BEIC’s British Lion Scheme, said researchers.”

A National Control Plan for *Salmonella* in poultry flocks in the EU was introduced in 2008.

The National Control Program^{16,17} in the UK and EU specifies sampling for *Salmonella* monitoring. Briefly this requires:

- Sampling chicks on arrival from the hatchery (chick paper, dead chicks)
- 2 weeks prior to move to the layer house (boot swabs or composite faeces)
- Layers between 22 and 26 weeks of age (boot swabs or composite faeces)
- Every 15 weeks during production. Official samples collected yearly.

If a flock tests positive for a regulated strain (SE or ST), the following occurs:

- Official confirmatory samples collected (no charge)
- Eggs* cannot be sold as Class A but can be sold as Class B (see below)
- Inspection of farm
- Additional testing to rule out false positive (if suspected false and no human cases linked) – extensive tests required (300 hen carcasses, 4000 eggs, dust and boot samples).
- If only dust samples are positive, repeat faecal sampling fortnightly (4 times) – If these are negative, the farm is considered negative.
- No official action is taken with SE or ST positive flocks due to the difficulty in clearing infection from laying houses.

*Class A eggs have normal shape, an air cell less than 6 mm, a yolk without a clear outline and is mobile within the egg, white is translucent, no foreign bodies and no embryonic development. They must not be washed or cleaned and must not be treated for preservation or stored below 5°C. Class B eggs do not meet these thresholds. Class B eggs are broken out or pasteurised.

The original successful approach to control of SE in layer flocks was established in the UK in the early 1990’s with the development of the Lion Brand program. Human cases of salmonellosis in the UK declined¹⁸ from 2.2 cases per 1000 person-years in 1996 to 0.7 cases per 1000 person years by 2012. The Lion Brand approach has developed into the “Laid in Britain” scheme¹⁹ by 2021. This is a voluntary program in UK.

The basics of the Laid in Britain program are briefly listed below. There is much more detail, but these are the main relevant points. For details, refer to the cited reference¹⁹.

- A documented plan for control on a layer farm, reviewed annually
- Staff training in compliance with the plan
- Pest control (red mite, litter beetle, flies and rodents)
- Housing standards for hygiene, heat stress control and terminal hygiene
- Specific housing for non-cage systems
- Feed and water standards – feed suppliers provide a warranty
- A plan if *Salmonella* detected in raw ingredients or feed
- Flock health plan, including a named veterinarian

- Biosecurity and disease control – includes not allowing permanent or long-term standing water on the farm; separated from other livestock
- *Salmonella* monitoring – sampling pre-placement, chick boxes and DOA's, regularly through life, enhanced once vaccination duration considered waned.
- *Salmonella* VACCINATION against SE and ST. Higher risk sites require live and inactivated vaccine.
- Anti-*Salmonella* products used must not compromise vaccination.
- Antimicrobials or other products to control *Salmonella* MUST NOT be used against *Salmonella* (masking effect)
- Egg handling – dirties and cracks removed. Washing eggs prohibited by law.
- Depopulation rules
- Packing station hygiene and maintenance.

Annex B of the Laid in Britain Code of Practice states the following in the event of detection of *Salmonella* Enteritidis on a farm:

In the event of non-vaccinal *Salmonella* Enteritidis (SE) or *Salmonella* Typhimurium (ST) being detected in faeces or on boot swabs or in the event of any other information becoming available that implicates the presence of *Salmonella* in a flock, Laid in Britain is to be informed immediately and the following action(s) is / are to be initiated:

- All eggs from the affected house on farm or in the packing station should be quarantined immediately. No further eggs should be sent to off-site packing stations. All eggs are to remain quarantined and not be released until notified by Laid in Britain.
- The private laboratory is to inform APHA and forward the *Salmonella* isolate at the earliest opportunity.
- Whilst waiting for the results from APHA, the retained veterinary surgeon is to carry out intensive sampling across the whole site. The samples should be processed in as timely a manner as possible and reported to Laid in Britain immediately.
- APHA to inform producer and Laid in Britain of the final results.
- If results are confirmed as non-vaccinal SE or ST, the producer, in conjunction with Laid in Britain, will decide whether to request a retest by APHA, if a false positive result is suspected. If the site is not to be re-tested, the producer should arrange for the slaughter of the birds immediately. Any eggs either on site or in the packing station should be destroyed or sent for heat treatment. No eggs should be packed as "Laid in Britain"
- Should a re-test by APHA be confirmed as positive for SE or ST, the birds should either be slaughtered, or all eggs should be sent for heat treatment. No eggs should be packed as "Laid in Britain".

Under this scheme, the farm loses its Laid in Britain status, but depopulation is not mandatory, and egg products can be used with heat treatment.

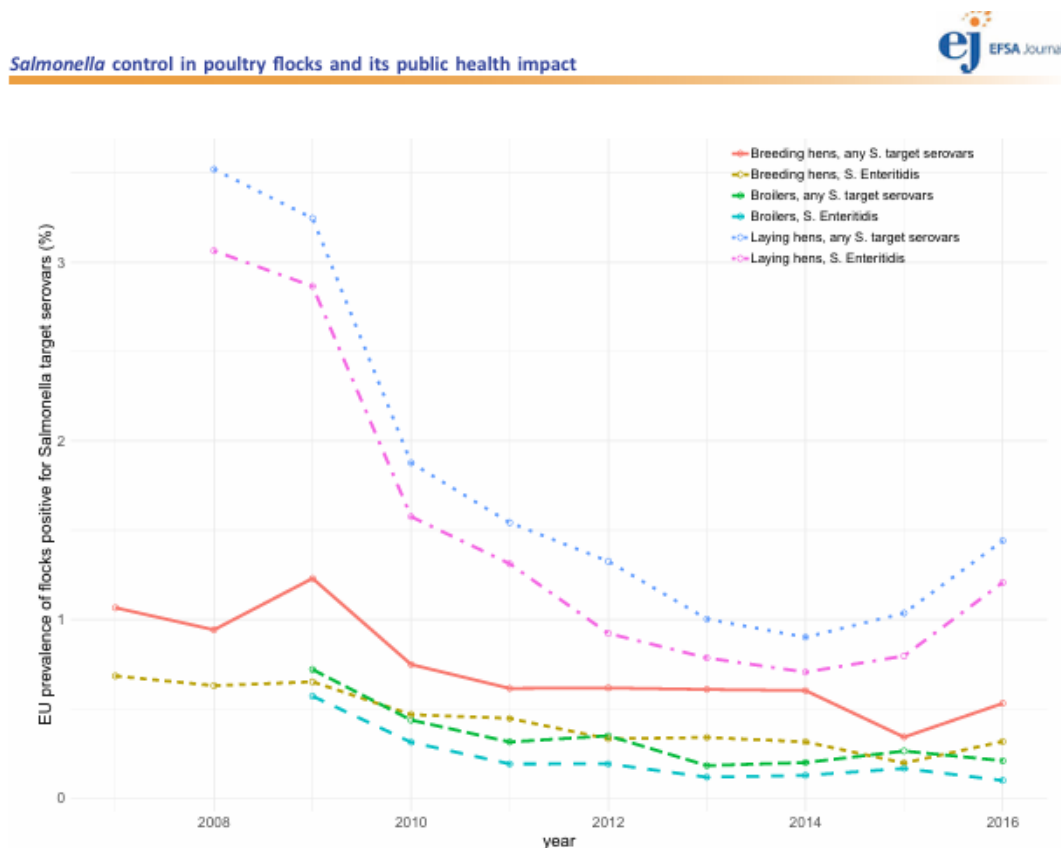
Follow up activities include traceability over the flock's life, *Salmonella* vaccination review and auditing of the next flock's vaccination procedure. Subsequent flocks receive both live and killed vaccines. A robust cleaning program is instituted, audited and site test negative before repopulation.

Europe

The European Food Safety Authority²⁰ investigated the effectiveness of *Salmonella* vaccination programs and found that 57.1% of human cases in the EU were attributed to *Salmonella* Enteritidis (SE), with the inclusion of *Salmonella* Typhimurium (ST) bringing the total to 75.9%. While SE cases increased in 2016 compared to 2014–2015, the rise was not statistically significant. From 2008 to 2016, SE cases in humans declined but stabilized between 2012 and 2016.

Between 2014 and 2016, SE remained the most prevalent serovar in human cases linked to eggs and poultry meat in the EU. A significant portion of these cases was attributed to international trade in table eggs, particularly from Poland and Germany, with contamination at the laying farm identified as the primary source. SE poses a challenge due to its ability to be transmitted vertically, leading to the presence of the organism inside the egg. This risk is heightened when eggs are consumed raw or lightly cooked.

SE prevalence in the EU declined from 2007 to 2013 but began increasing in both layer flocks and human cases thereafter, alongside a rise in *Salmonella* Infantis and other serovars²⁰. The prevalence of *Salmonella* in breeder flocks was reported as 0% in three EU countries²⁰, with the overall EU breeder flock prevalence decreasing from 1.12% in 2007 to 0.32% in 2016.



Source: Overall prevalence of *Salmonella* target serovars in poultry populations in the EU, 2007–2016. (EFSA, 2019).

SE in breeder flocks in 2016 in the EU showed positive detections from flocks in Poland, Greece, France and The Netherlands, while other countries, including the UK, reported 0% SE detections in breeders.

Control in UK and Europe following introduction of SE vaccination in layers has been hugely successful. “By 2012, figures showed that, in the UK, the level of *Salmonella* of public health significance in laying flocks had fallen to 0.07% and it has remained low. In 2016, the UK Food Standards Agency announced a review of its advice on egg safety following an official report which concluded that British Lion eggs could now be safely eaten runny, even by pregnant women, babies and elderly people.”

USA

Control approaches vary somewhat by states in the USA²¹. To quote this publication: “Over 60 percent of farms in the Northeast and West regions participated in a State egg (*Salmonella* Enteritidis) quality assurance program, compared with less than 10 percent of farms in the Southeast and Central regions; the majority of farms in the Southeast and Central regions participated in company sponsored programs. A higher percentage of farms in the Southeast and West regions participated in a commodity-group quality assurance program compared with farms in the Northeast and Central regions.”

“A higher percentage of farms in the West region than in the other regions would likely take hens out of production if *Salmonella* Enteritidis were to be found in the layer house environment. Farms in the Northeast and Southeast regions were more likely to culture and divert eggs if *Salmonella* Enteritidis were to be found compared with farms in the Central and West regions.”

Across all five regions, the proportion of farms likely to take hens out of production varied from 14.8% to 69.5% if SE were detected early in the production cycle compared with 30 to 76% that would take hens out if SE was detected late in the production cycle. A higher percentage of small farms than large farms (39.7 and 19.6 percent, respectively) would likely remove hens from production if SE were to be found in the layer house environment. At any stage, 70 to 98% of farms that detected SE would divert eggs from table consumption until the farm tested culture negative.

The percentage of flocks testing positive to SE in the five USA zones over 2012-13 ranged between 0.3 to 1.2%.

SE vaccination is well established in USA. In 2013, 99.6% of US layer farms with less than 30,000 birds, 100% of farms with 30,000 to 99,999 birds and 97.9% of flocks with 100,000 or more birds were vaccinated against several serovars of *Salmonella*, including Enteritidis.

A report²² described trends in human salmonellosis related to 12 commodity groups in the USA. The trend for egg-related proportion of total outbreaks significantly decreased from 0.33 in 1998 to 0.06 in 2017, even though the total number of egg-related outbreaks increased (thus the total number of outbreaks due to non-egg sources increased proportionally more over this period).

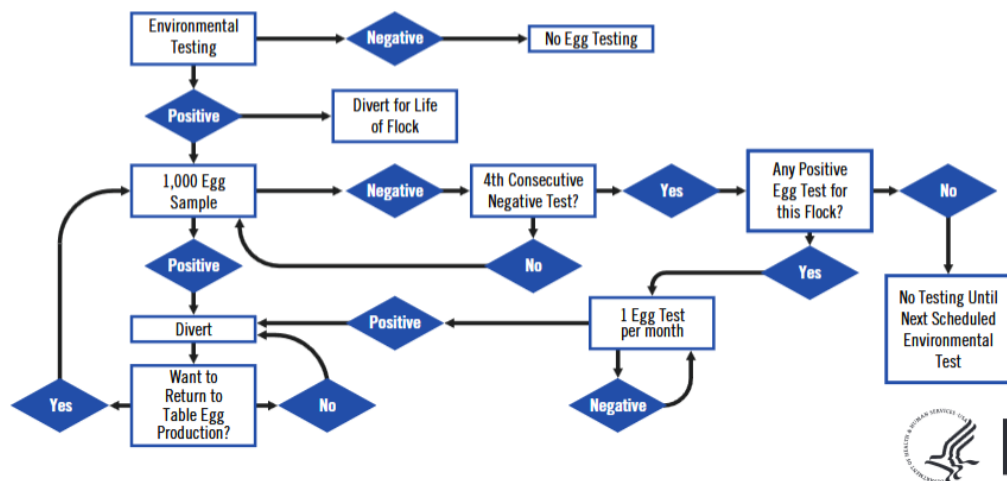
The USA FSIS Roadmap to reducing *Salmonella*²³ (2020) specifies that “FSIS inspectors verify that establishments implement food safety systems that reduce *Salmonella*, and poultry establishments are categorized based on their test results for each commodity produced. Establishments that meet the performance standards are classified as either category 1 or 2. If an establishment falls into category 3 (not meeting the performance standard), the Agency assigns a PHRE, during which FSIS investigators evaluate whether the establishment has adequately addressed *Salmonella* in the food safety system. The establishment must take corrective actions and may also need to reassess and modify food safety programs to adequately address *Salmonella*. FSIS employees may also perform an FSA to investigate any existing vulnerabilities in the food safety system.”

USA procedure in the event of SE detection:

The US Food & Drug Administration outlines “The Egg Rule” as to the choices a farm can make in the advent of a detection of SE²⁴.

Under this approach, a farm with a positive SE detection from a routine monitoring sample of the birds or their environment can manage the situation independently. The farm has a choice of (a) testing the eggs (see below) and adopting additional mitigation practices; (b) diverting all eggs to pulp or pasteurization; or (c) depopulating the flock. Under the US approach, the flock does not have to be destroyed, although the farm can decide for this option.

The farm can undergo the “Egg Test” protocol which involves four consecutive samples of 1000 eggs to be tested by culture for SE (pooled in groups of 20; hence 50 cultures) at 2-weekly intervals. If no positive tests are obtained from the eggs, the flock is allowed to resume supply of table eggs with only routine environmental monitoring thereafter (providing this is negative). If, however, any of the egg tests are positive, eggs remain diverted, and the flock can undergo the process again if it wishes to return to table egg production. A flow chart of the process is reproduced below. This process also requires a written SE prevention plan including refrigeration of eggs under 7°C. Cleanout after a flock has been positive is only required to “remove all visible manure, dry clean the house and disinfect with spray, aerosol, fumigation or another appropriate disinfection method”.



The Egg Rule used in USA (FDA, 2022)



Processes through which authorities could maintain confidence that effective SE management were in place such that depopulation would not be required in response to a detection of SE

Technical issues

Most human salmonellosis cases are caused by SE (overseas at least) and *S. Typhimurium*. (ST). SE shares the ability of the chicken-adapted serovars (*S. Gallinarum* – fowl typhoid; and *S. Pullorum* – Pullorum disease) in an ability to colonize the reproductive tract of mature hens. ST has a lesser ability to do this and most of the other serovars (e.g. *Infantis*, *Mbandaka*) mostly contaminate the eggshell via cloacal contact during laying. The main site that *Salmonella* reside in the chicken however is in the blind gut pouches (caeca). Research⁹ has shown that intestinal environments are hostile to *Salmonella*. *Salmonella* must compete with a complex gut microbiota, the bird's immune response and many metabolic materials (e.g. butyrate) present in the caeca.

There is an initial inflammatory response to infection, but this does not produce overt disease in the chicken, but the bacteria can temporarily make its way to the liver and spleen and, in some cases, to the reproductive tract.

The gut flora effectively competes with the pathogen and thus we have many probiotic and prebiotic products on the market aimed at *Salmonella* minimization in the gut.

Wigley⁹ pointed out that an ability to colonize the reproductive tract by bacteria is very challenging. SE is able to achieve this, however. It has been shown⁹ that vaccination is highly protective of the reproductive tract of hens, via antibody and cell mediated responses.

However, there is a particular weak point that we find for the hen, and this occurs at sexual maturity. As egg production starts, the hen temporarily switches off her cell mediated immunity. This allows previously controlled organisms in the gut to begin to grow and spread with this drop in immune protection from the hen. This has been shown for *S. Pullorum*²⁵ and for SE²⁶ and we have produced good evidence that it also happens with ST²⁷. This is a vital point. Susceptibility to infection of the reproductive tract by SE is increased at point-of-lay²⁶. It also explains why many other organisms (viruses, like Chicken Anaemia Virus, Avian Encephalomyelitis virus and Avian Adenoviruses and bacteria like *Mycoplasma*) that have infected the hen months before and been held in check by the immune system, can re-emerge at point-of-lay and be passed onto the chicks through the egg.

Why would the hen do this? It doesn't happen in the rooster.

This is an evolutionary development that is designed to add protection to her chicks. She shuts down her cell mediated immunity, which mostly suppresses organisms that live inside her cells, still present but well inhibited. This allows these suppressed organisms to multiply at this time. However, her antibody producing system is still active, so this surge in organisms will stimulate strong antibody production. This antibody in her blood is transferred into the developing egg yolk – the result is a heap of antibody in the yolk to provide passive protection to the newly hatched chick. So, the hen puts herself at risk to increase protection to her chicks. This also happens in mammals, encouraging

antibodies to be included in colostrum to achieve the same effect.

But this process also lets the pathogenic organisms have a chance. If say, the pullet was infected by a *Salmonella* early in life, and she suppressed it, it may suddenly burst forth at point-of-lay, in a flock that is synchronized to have low immunity at this time. So, even if only a very few hens were infected, the increased excretion of the *Salmonella* could gain access to many susceptible birds. Even if they were previously vaccinated with a live vaccine that promoted cell mediated immunity, they may again be susceptible at this point²⁶. The bacteria will spread and establish in the flock. But, if the birds have been vaccinated to produce high levels of antibody to *Salmonella* (e.g. by a killed vaccine or injected live one), then the re-surgings *Salmonella* will be inhibited and not be able to establish again in the flock²⁸. The existing presence of antibodies in her blood will also be protective against bacterial spread to the reproductive tract.

This observation is common to many experimental studies with SE. A systematic literature review²⁹ analysis of infection studies with SE found that the most susceptible time of infection in layer hens occurs around sexual maturity (28 studies included in this review).

A study with ST in Australia³⁰ showed that ST excretion in faeces declined after 5 weeks but it continued to be shed in faeces intermittently at low levels for long periods of time and that the organism could be found in the reproductive tract. However, in that study, ST was found on the shell but was not able to be detected inside eggs.

Antibody in the egg yolk also inhibits *Salmonella* growth⁹.

An Australian Eggs funded study by Scott et al.³¹ showed that although intestinal/ faecal levels of SE could not be eliminated by administration of live ST vaccine followed by an inactivated SE vaccine, colonization of the ovary was prevented.

A model³² proposed in 2014 estimated that vaccination facing variant strains of ST may not reduce faecal shedding but still significantly reduced the rate of positive egg contents and visceral organ infection

Vaccine use

Logue et al.³³ recently comprehensively reviewed *Salmonella* control interventions in poultry meat production. A major focus in the broiler industry is protection of the breeder birds, to prevent vertical transmission of *Salmonella* through or on the egg. This is an identical goal for egg layers, but with the latter the object is protection of the consumer. The processes however are identical. This review notes that the hatchery is the most significant source of *Salmonella* for broiler chicks. This reflects vertical transmission (inside the egg or on the eggshell) as well as contamination of chicks horizontally within the hatchery operation and this emphasises the role of the egg for human infection. The review notes the importance of hygiene and biosecurity in limiting the entry and establishment of *Salmonella* from outside sources, which is also practiced on layer farms. The report³³ highlights the aims of vaccination against SE and ST as to prevent infection of the reproductive tract and to reduce faecal excretion of the organisms.

The Logue review³³ describes the vaccine types available overseas. Live vaccines are mostly mutant strains with deleted genes. Vaxsafe® ST (Bioproperties) and Poulvac® ST (Zoetis) are live *aro-A* deletion mutant vaccines available in Australia. Overseas there are a larger variety mutants based on deletion of other genes. It is noted that these strains need to remain in the hen's cells long enough to induce immunity but not persist in the environment. They are usually administered orally or by spray and are hoped to become dominant in the gut, competing with wild strains that may challenge the bird. They are intended to induce both cellular and antibody immunity. The live *aro-A* mutants

available in Australia, however, do not provoke antibody responses if given orally or by spray. There are some claims that the live vaccines provide cross immunity against other serovars, such as SE, but this is noted as “inconsistent”. Studies with an *aro-A* mutant in Australia against the NSW strain of SE provided only partial protection when the vaccine was administered by injection, twice (Clark et al., unpublished).

There is mention of an *aro-A* deletion SE mutant available overseas, but this is not available in Australia at present.

Live vaccines (at least those available overseas) are claimed to provide better protection than inactivated vaccines, as they induce the cell mediated response, whereas inactivated (killed) vaccines predominantly produce antibody. Logue’s report³³ notes that in chicken breeder flocks it is common to give a live vaccine followed by an inactivated vaccine. They also observe that antibody levels in hens had a direct effect on inhibiting *Salmonella* colonization of internal organs, most importantly, the reproductive tract. The usual approach in an increased *Salmonella* mitigation program that would be adopted in USA layer flocks that had SE detected would entail a live vaccination at the hatchery (Megan Vac®), a live ST vaccine at 3-5 weeks (Poulvac® ST), another live vaccine at 8-9 weeks (Megan Vac®) and an inactivated SE vaccine at 10-14 weeks (Dr M. Bland, pers. comm.)

An Australian study³⁴ showed that two injections of STM1 (*aro-A* deletion ST mutant) caused persistent antibody levels during lay but this was unable to prevent the environmental or faecal presence of *Salmonella* serovars (including ST) in the layer environment in early lay, supporting the association with cell mediated immunosuppression at sexual maturity explained above. No egg contents were found to be positive for ST, however.

Also available overseas are subunit vaccines (made from a piece of the bacteria only) and these report higher antibody and cellular immune responses.

What can we do in Australia?

Biosecurity, farm hygiene, rodent control, and egg handling have been addressed in our situation, in many ways similar to overseas practices. The missing piece in our armoury is an SE vaccine.

The availability of an effective vaccine that covers SE, ST and perhaps *S. Infantis* would make a huge improvement in the ability to control *Salmonella* in Australian poultry flocks.

This should give a boost to confidence to the farmers and the Food Authority in protection against SE being able to contaminate eggs. With an incrementally increased protection against SE establishment in flocks, the need for an eradication approach in the case of a detection is decreased. The risk of SE establishing in a vaccinated flock and the chance of egg contamination would be substantially reduced.

The overall goal of such a vaccination strategy would be to drastically reduce, if not eliminate, the likelihood of a SE shedding through the reproductive tract (i.e. inside eggs).

This would provide a strong protection of the consumer and remove the fear of financially ruining farms that may find SE in their environment. The addition of other serovars to the inactivated vaccine would strengthen protection against ST, currently the major serovar of concern in Australia³⁵, and other emerging serovars like *Infantis*. Other serovars could be added if they rose in prevalence in poultry populations.

An improved framework for effective SE management in Australia would include:

- Maintaining current on farm SE mitigation practices.

- Making inactivated SE + ST (+SI ?) vaccines available for widespread use;
- Setting up an audited quality scheme involving mandatory vaccination; and
- removing the need for participating farms to dispose of impacted flocks.
- procedures for the use of eggs where SE is detected in this status of flock –that allows the choice of either sending all eggs to pulp or testing eggs under an approved program to determine if the eggs are free from SE – if so, eggs can be used as normal.
- An adequate farm cleanout procedure before the next bird batch introduced to the property is only required; no requirement for demonstration of complete freedom as long as the next batch has been fully vaccinated.

Farms adopting an authorized vaccination plan against SE should be able to follow similar response initiatives as overseas countries allow.

Regulatory and commercial pathways to securing supply of effective SE vaccines in Australia

There are currently no readily available SE vaccines in Australia.

An autogenous SE vaccine would require that an SE is found on a property and that a laboratory or vaccine manufacturer who isolated the organism could manufacture it into an inactivated vaccine which could then be used on the property from which it originated and possibly properties with a strong epidemiological link to that property. But this requires your farm to be wiped out first and be able to prove freedom to the current requirement.

A better approach would be to ask a laboratory or vaccine manufacturer to produce an inactivated SE vaccine (preferably also containing ST and SI) and apply for a minor use permit, allowing it to be sold under the terms of that permit. This would allow the industry to use it as long as there was no registered alternative vaccine fit for this purpose. This is how standard minor use permitted inactivated fowl cholera and infectious coryza vaccines are currently available.

The manufacturers of the registered live ST vaccines may object however, as they cover ST. Hence the minor use permit would only cover an SE vaccine (or perhaps SE plus SI) to differentiate the usage. Still the registered ST vaccine producers may object as there is some partial cross protection against SE.

Perhaps an answer to this problem would be to encourage, or even mandate that the live ST vaccines could be used as a primer dose(s), to be followed by one or two inactivated SE vaccine doses, as proposed in the work by Scott et al.³¹ which was funded by Australian Eggs.

This would be an interim approach until a fully registered SE vaccine could be made available.

The proposal would need to be supported by the Australian poultry industries (Australian Eggs Limited and ACMF would need to support the application, and a majority of Australian poultry veterinarians). The industry would need to assure the vaccine supplier(s) that this product would achieve adequate sales to cover the high registration costs and the support of authorities overseeing SE response processes would be essential for this.

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