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Supporting document 1

Microbiological risk assessment of *Salmonella* in eggs – Proposal P1060

Egg Food Safety & Primary Production Requirements

EXECUTIVE SUMMARY

This microbiological risk assessment addresses the public health risks associated with consuming eggs and egg products in Australia. It provides an objective interpretation of available scientific data and identifies key microbiological food safety hazards. It assesses where in the primary production and processing supply chain these hazards may be introduced, increased, reduced or eliminated.

When Standard 4.2.5 was developed in 2012, *Salmonella* Enteritidis (SE) was not present in Australian layer flocks. The standard did not incorporate measures to address vertical transmission of SE into the egg and potential for subsequent growth of SE in intact eggs.

The status quo has changed and SE infections are occurring in Australian layer flocks and causing human illness. The 2018-19 outbreak and subsequent sporadic occurrences of SE confirms current Australian requirements to manage SE risks in eggs do not effectively protect public health and safety.

This risk assessment confirms additional management measures are required in the Code to prevent human illness resulting from consuming SE contaminated eggs. Qualitative risk assessment and quantitative modelling are used to assess the risk and evaluate potential illnesses with and without proposed management measures.

Qualitative assessment

A combination of multiple strategies can be used to control SE risk, including biosecurity measures, vaccination, feed additives, animal and pest control, farm hygiene, environmental monitoring and egg refrigeration. The measures a business adopts needs to be considered within the entire system of controls; ineffectiveness of one measure can impact effectiveness of the whole SE management system.

There is a strong body of evidence on effective SE control measures given SE is endemic in many countries. Supplementing this evidence with information from recent Australian outbreaks, FSANZ concludes on-farm monitoring for SE and temperature control of eggs through chain as part of the management system, will reduce SE related foodborne illness. These activities must be supported by enhanced on-farm hygiene and biosecurity measures.

Further, enhancing traceability requirements will provide faster traceback to a source farm and stopping supply of potentially SE-positive eggs from entering the human food supply.

Quantitative model

The quantitative model simulates through-chain stages of egg production, distribution and consumption, estimating the likelihood of contamination and subsequent illness under different scenarios. Further, the model allows investigation of interventions such as environmental testing, passive human surveillance (PHS) and temperature control at different parts of the supply chain.

Implementing on-farm environmental testing reduces the number of illnesses associated with SE-positive egg layer farms, on both small (1,000 hens) and medium (20,000 hens) sized farms, with more impact for medium sized farms. Without this monitoring the majority of small farms would go undetected because the number of notified illnesses are not high enough to trigger successful epidemiological traceback investigations. While a single test during production testing shows a decrease in SE illness, the most effective testing schedule is at regular 13-week periods. Regular testing is more likely to detect SE on-farm prior to illnesses occurring. A single test during a flock's production cycle is not as efficient as regular 13-week testing at protecting public health, and may not lead to more illnesses avoided than relying on PHS. This result is true for both small and medium sized farms but has increased impact for medium size farms.

Refrigeration of eggs from both small and medium size farms, through the supply chain greatly decreases human illness. Refrigeration prevents growth of many microorganisms including SE if present in egg contents. A farm can have more confidence in preventing foodborne illnesses when through-chain refrigeration is in place.

When implemented in tandem, environmental testing and refrigeration show the greatest decrease in SE illness.

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GLOSSARY

Dose response – Dose-response refers to the relationship between the amount of exposure (number of microorganism) that leads to health or biological effects.

Exposure assessment – The qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant.

Hazard characterisation – The qualitative and/or quantitative evaluation of the nature of the adverse effects associated with biological, chemical and physical agents which may be present in food. A dose response assessment may be undertaken if appropriate data is available and it is necessary to address the questions asked by risk managers.

Hazard identification – The identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Potentially hazardous food (PHF) – Means food that has to be kept at certain temperatures to minimise the growth of any pathogenic microorganisms that may be present in the food or to prevent the formation of toxins in the food.

Qualitative risk assessment – A process used to evaluate risks based on subjective judgment and qualitative data rather than quantitative data. It involves identifying, analysing, and prioritising risks in order to make informed decisions about how to manage and mitigate them. This type of assessment is often used when there is limited or incomplete data available, or when a more in-depth analysis is not feasible or necessary.

Quantitative risk assessment – A systematic and structured process used to assess and quantify risks associated with various hazards or potential events to make informed decisions and prioritise risk management strategies. Numerical data and models are used to estimate the likelihood and potential consequences of adverse events, providing a more objective understanding of risks.

Risk characterisation – the process of determining the qualitative and/or quantitative estimation, including uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment.

Risk factor - A characteristic or condition that contributes to the probability of a negative health outcome.

Risk profile – The description of the food safety problem and its context.

Yolk mean time (YMT) – Time to break down the membrane (known as the vitelline membrane) that separates the egg white from the yolk. This membrane prevents pathogens like *Salmonella* Enteritidis entering the nutrient-rich environment of the yolk and growing exponentially.

ABBREVIATIONS

AECL	Australian Egg Corporation Limited
CFU	colony forming units
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FMM	Food Ministers Meeting
FRSC	Food Regulation Standing Committee
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
NSWDPI	New South Wales Department of Primary Industries
NSWFA	New South Wales Food Authority
PHF	potentially hazardous food
SAGE	Scientific Advisory Group for Eggs
SDAG	Standards Development Advisory Group
SE	*Salmonella enterica* subspecies *enterica* serovar Enteritidis
ST	*Salmonella enterica* subspecies *enterica* serovar Typhimurium
USDA	United States Department of Agriculture
WHO	World Health Organization
YMT	yolk mean time

BACKGROUND

In October 2020, FSANZ started project W1138 – Review of the Egg Primary Production and Processing Standard to review through-chain requirements for egg production in Australia. FSANZ concluded a proposal was necessary to manage egg-related food safety risks. Key issues to be considered include:

- stronger requirements on bird health and through-chain traceability (including egg stamping)
- definitions and requirements on unsuitable eggs and egg washing, and
- through-chain temperature control of eggs.

Proposal P1060 – Egg Food Safety and Primary Production Requirements commenced in May 2022.

The primary hazard considered is *Salmonella enterica* subspecies *enterica* serovar Enteritidis (SE) and the emerged risk to public health and safety following its detection in Australian layer farms in 2018–2019. The risk from other *Salmonella* serovars, notably *Salmonella enterica* subspecies *enterica* serovar Typhimurium (ST) is also considered.

Previous egg proposal P301
Standard 4.2.5 was developed through Proposal P301 – Primary Production of Eggs and Egg Products. The FSANZ scientific risk assessment answered three key questions:

(1) What are the microbiological and chemical risks to food safety posed by the consumption and use of eggs and egg product in food in Australia?

(2) Where during the production and processing of eggs and egg product may hazards be introduced and/or their levels change, and which factors have the most significant impact on public health and safety?

(3) What are the hazards and subsequent risks associated with emerging pathogens such as SE and highly pathogenic avian influenza?

The 2011-12 P301 risk assessment found *Salmonella* spp. and specifically ST was the hazard of greatest concern for eggs in Australia. There was limited epidemiological evidence implicating clean, intact eggs as a source of egg-associated foodborne illness outbreaks. Reported outbreaks were generally attributed to the consumption of uncooked foods containing raw egg (e.g. desserts and egg-based sauces). A common risk factor identified in outbreaks was the use of eggs with visible surface faecal contamination (dirty eggs). Contributing factors included cross-contamination during food preparation and/or temperature abuse of food containing raw egg after preparation.

Risk factors identified as having potential to introduce *Salmonella* spp. into a laying flock include feed, water, pests (rodents and insects), environment, personnel, new laying stock and equipment. Many of these factors can be managed through biosecurity programs, which aim to reduce transmission of avian diseases into layer flocks. As there are multiple ways for *Salmonella* spp. to be introduced into layer flocks, FSANZ did not rank the importance of factors in P301.

Key risk factors for horizontal transmission of *Salmonella* spp. into egg contents are presence and load of external contamination (e.g. faecal material), temperature differential

between the egg and environment especially at point of lay, humidity, and condition of the shell (e.g. cracks), cuticle and membranes.

P301 found *Salmonella* serovars that can colonise the ovaries of hens and directly internally contaminate eggs prior to lay (i.e. vertical or trans-ovarian transmission) were not endemic in Australian breeder or layer flocks and had not been associated with an outbreak.

The final assessment report noted Standard 3.2.2 – Food Safety Practices and General Requirements requires potentially hazardous foods to be stored under temperature control. FSANZ's guide to Standard 3.2.2, *Safe Food Australia*, states intact eggs in Australia are not considered potentially hazardous foods because they are unlikely to be infected internally with *Salmonella* spp.; at that time FSANZ concluded there was no need to refrigerate eggs to prevent bacterial growth.

Changed risk profile for eggs

The SE incident in 2018–2019 changed the risk profile for Australian eggs.

Further, through Proposal P1053 – Food Safety Management Tools (2021–2023), raw eggs when handled by food service and retail sectors were assessed as being a high risk, potentially hazardous food.

Where egg contents or the inside of a whole egg contains a pathogen such as *Salmonella* Enteritidis, the egg is a potentially hazardous food.

1 INTRODUCTION

This microbiological risk assessment addresses the public health risks associated with consuming eggs and egg products in Australia. It provides an objective interpretation of available scientific data and identifies key microbiological food safety hazards. It assesses where in the primary production and processing supply chain these hazards may be introduced, increased, reduced or eliminated.

1.1 Risk assessment questions

The FSANZ risk assessment responded to the following questions:

Q1. How has the food safety risk changed for eggs since the risk assessment for Proposal P301?

Q2. What on-farm practices, risk factors and controls would address the new food safety risks for eggs?

Q3. For supply chain management, when do eggs become potentially hazardous and how would this be managed?

1.2 Development and structure of the risk assessment

The questions posed are complex and interrelated, and require both qualitative and quantitative assessment. This risk assessment uses information from P301 relevant to both SE and non-SE *Salmonella* serovars, supplemented with more recent scientific literature.

Questions about through-chain egg temperature or storage time are best answered using quantitative models of egg supply chains. These models can evaluate scenarios and changes in risk associated with consuming eggs and food containing eggs. The P301 risk assessment used the results for a quantitative model developed for Australian Eggs Corporation Limited (AECL) (Thomas et al., 2006). This model was subsequently modified by the European Food Safety Authority (EFSA) to evaluate spoilage of eggs in Europe (EFSA, 2014). The EFSA model was further developed by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and German Federal Institute for Risk Assessment (BfR) (Desvignes et al., 2019). The ANSES/BfR model has now been adapted for Australian egg supply chains under this proposal (refer to SD2).

Following the Codex Alimentarius Commission (1999) Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment, which applies to risk assessment of microbiological hazards in food, the structure and content of this report is indicated below:

- section 2 hazard identification: using evidence gathered during the 2018–2019 SE incident and epidemiological information for other outbreaks of SE and ST;
- section 3 hazard characterisation: describing how estimated SE levels in a serving of food were used to estimate the probability of illness;
- section 4 exposure assessment: describing how SE gets on farm and into the egg supply leading to consumer exposure;
- section 5 risk characterisation: providing estimates for the likelihood of illness from SE in eggs in supply chains (uses outputs from the quantitative risk model described in SD2).

    - This model focuses on egg supply chains for small (1,000 hens) and medium (20,000 hens) egg layer farms. Models for large (100,000+ hens) egg layer farms were not considered necessary because large farms are already on voluntary (or in NSW mandatory) schemes. These schemes include SE management strategies similar to those proposed under P1060, including regular environmental monitoring to maintain accreditation.
- section 6: identification of data gaps, assumptions and uncertainty in our modelling;
- section 7: responses to risk assessment questions, including information on the efficacy of alternative or combinations of scenarios in mitigating the risk of illness; and
- section 8: references
- section 9: annexes for additional quantitative model outputs and data gaps elaboration

2 HAZARD IDENTIFICATION

Summary of hazard identification

There are over 2,500 serovars of *Salmonella enterica* and almost all are capable of causing salmonellosis. *Salmonella* multiplies in food products if temperatures during storage and transportation allow. *Salmonella* grows in temperatures ranging from 5-46°C, with optimal growth at 37–42°C. It is usually destroyed when food is prepared at temperatures exceeding 70°C. It thrives in mildly acidic to neutral pH levels and is inhibited by high salt concentrations and low water activity. *Salmonella* can survive in various environments like water and soil by entering a dormant state, making detection challenging. *Salmonella* can form biofilms, protecting it from external stresses. It uses a type III secretion system to inject proteins into host cells, aiding invasion and survival.

Salmonella is a leading cause of bacterial diarrheal disease globally, causing gastroenteritis. The severity varies with host factors and serotype. Children, the elderly, and immunocompromised individuals are more likely to experience severe symptoms. Australia has high salmonellosis notification rates, with 72% of cases considered foodborne. Despite reduction efforts, the incidence remains high. Eggs and egg-containing foods are frequent causes of *Salmonella* outbreaks in Australia.

Salmonella can be transmitted vertically and horizontally within poultry flocks. SE-infected flocks are often asymptomatic, complicating detection and control. *Salmonella* infections in poultry can vary, with SE demonstrating higher frequencies of vertical transmission and internal egg contamination than other *Salmonella* spp. Within an SE-positive flock, the frequency of internally contaminated eggs being laid is low. However, over time a large pool of SE-contaminated eggs can be produced by a single farm.

Within Australia, ST is responsible for most egg-related outbreaks. Of these, most outbreaks occur in commercial food settings, with traceback investigations used to identify the farm of origin. The 2018-19 SE outbreak in Australia was linked to eggs and resulted in widespread illness, recalls and biosecurity responses. Following this SE outbreak, commercial flocks in Australia continue to test positive for SE. In addition to farms testing positive, human illness notifications involving the 2018 SE-incident strain continue to sporadically occur.

2.1 Salmonella *and salmonellosis*

The *Salmonella* genus consists of two species: *S. enterica* and *S. bongori*. Over 2,500 serovars of *S. enterica* are known. *S. enterica* is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica* (Grimont and Weill, 2007). Salmonellae are serotyped by identifying the O- and H- antigens (phase 1 and 2) to name the serovar. Names for *Salmonella* serovars have only been maintained for the subspecies enterica serovars, which account for more than 99.5% of isolated *Salmonella* strains.

Salmonellosis is caused by *S. enterica* bacteria and is one of the major zoonoses in many countries. Almost all serovars can cause infection in humans, although there are differences among serovars in terms of prevalence, transmission route and pathogenic potential.

2.2 Growth characteristics

Salmonella are facultative anaerobes capable of growing in both aerobic and anaerobic conditions. Its growth is influenced by several factors, including temperature, pH, water

activity, and nutrient availability. One of the key growth parameters is temperature. *Salmonella* can grow in temperatures of 5–46°C, although its optimal growth range is between 37°C and 42°C, which corresponds to the human body temperature (D'Aoust, 1989). This provides optimal temperature conditions for *Salmonella* to colonise the host's intestinal tract, causing gastroenteritis and other related illnesses. The minimum water activity for growth is 0.95, but *Salmonella* can survive for long periods in low moisture foods and dry material. *Salmonella* growth is prohibited by NaCl concentrations at 9% and above. *Salmonella* can tolerate a wide pH range, between 4.0–9.5, but tends to thrive in mildly acidic to neutral conditions (Li et al., 2012). This adaptability enables it to survive the acidic conditions of the stomach and establish infection in the intestines.

Salmonella bacteria can multiply in food products if the temperature during storage and transportation allows. *Salmonella* can survive under harsh conditions, including persisting in frozen meat for a year or more (Muller et al., 2012). *Salmonella* is usually destroyed at temperatures above 70°C, so it may persist in products processed at lower temperatures.

2.3 Survival mechanisms

Salmonella has evolved various survival mechanisms contributing to its persistence in diverse environments, such as its ability to form biofilms. Biofilms are complex communities of bacteria embedded in a self-produced matrix of extracellular polymeric substances. This protective matrix shields *Salmonella* from external stresses, including antibiotics and the host's immune responses (Finn et al., 2013). Additionally, *Salmonella* possesses a type III secretion system (T3SS), which is a critical virulence factor. The T3SS enables the bacteria to inject effector proteins directly into host cells, facilitating invasion and survival within the host (Matsuda et al., 2019). This mechanism provides *Salmonella* with a means to evade the host's immune system and establish a persistent infection.

2.4 Environmental adaptations

Salmonella is adept at surviving within hosts and also in various environmental niches (Brown et al., 2021). It can persist in water, soil, and a wide range of foods. The bacteria can endure harsh conditions, such as desiccation and extreme temperatures, by entering a viable but non-culturable state. This dormant state allows the bacteria to evade traditional detection methods, posing challenges for food safety and surveillance.

2.5 Public health significance of Salmonella

The growth and survival capabilities of *Salmonella* have significant implications for human health. *Salmonella enterica* is one of the most commonly reported causes of foodborne human gastroenteritis with an incubation period of 12–96 hours (Hernandez et al., 2012). Most cases of salmonellosis are mild and self-limiting, lasting up to a week; however, in some cases it can lead to hospitalisation and be life-threatening. The severity of disease depends on both host factors and the serotype of *Salmonella*. Salmonellosis is usually characterised by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. Children younger than five, the elderly, and people with weakened immune systems are more likely to have severe salmonellosis symptoms. Contaminated food products are frequently implicated in salmonellosis outbreaks (Moffatt et al., 2016). SE and ST have dominated the global epidemiology of *Salmonella* and are the most common causes of human salmonellosis (Hendriksen et al., 2011).

2.6 Salmonellosis notifications

Despite the introduction of Standard 4.2.5 in 2012, the incidence of salmonellosis

notifications in Australia steadily increased until 2016. Compared to the USA, Canada, UK and New Zealand, Australia still has one of the highest salmonellosis notification rates (Figure 1). A large proportion (72%) of salmonellosis notifications in Australia are considered to be foodborne (Vally et al., 2014). Other sources of exposure include environmental sources, contact with wild and domesticated animals, and overseas travel.

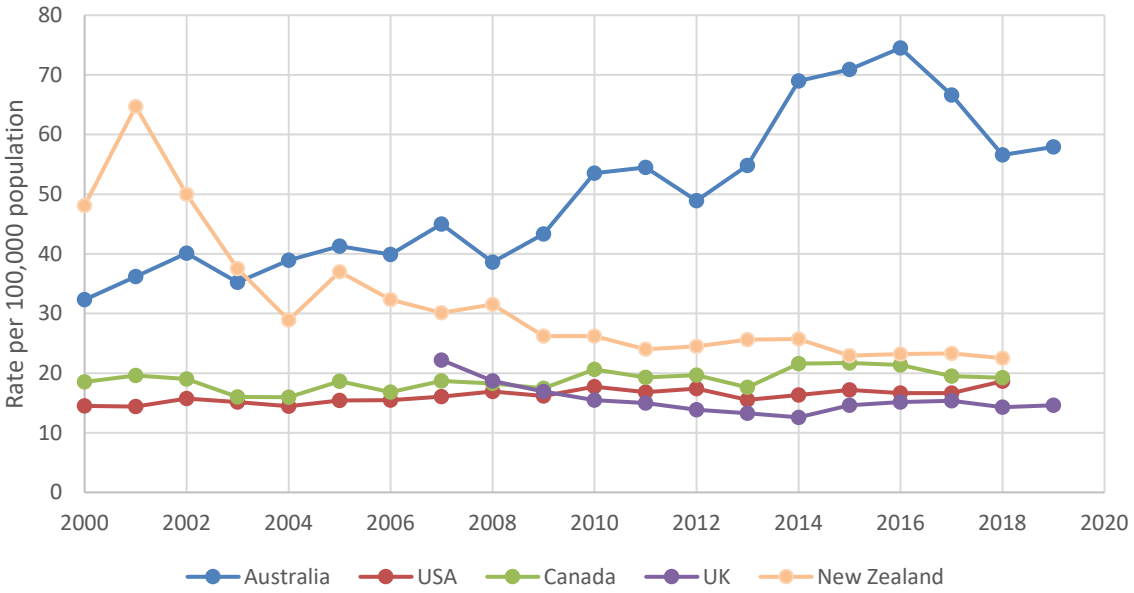

Figure **1***: Salmonellosis notification rates (all serotypes combined) in selected countries.*

Source: Australia – NNDSS; New Zealand – ESR Public Health Surveillance; Canada – Public Health Agency of Canada; UK – ECDC Surveillance Atlas of Infectious Diseases; USA – MMWR annual reports and CDC Wonder (2016-2018).

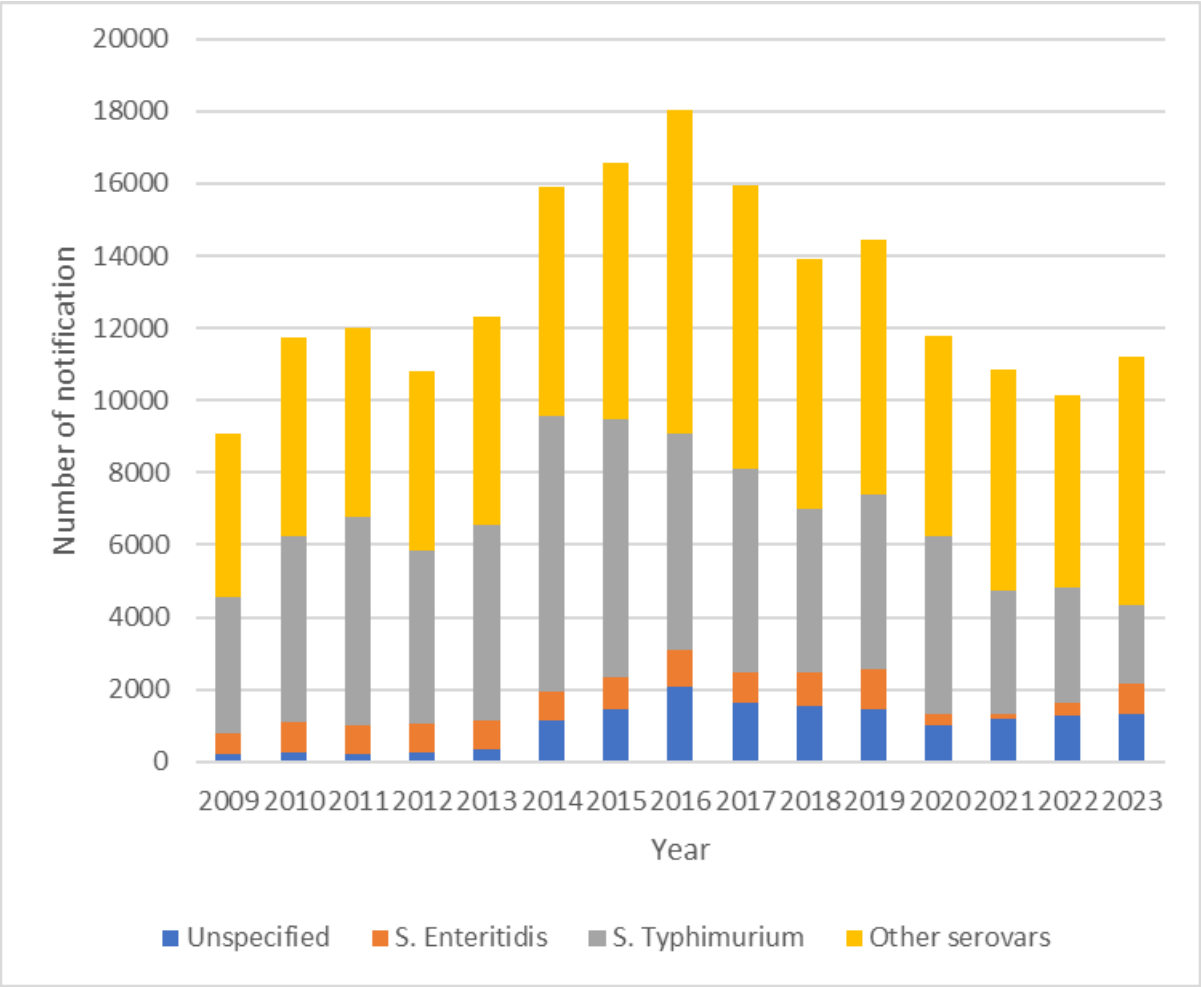

Figure 2: Australian salmonellosis notifications for S. Typhimurium, S. Enteritidis, serovar unspecified and all other Salmonella serovars. The increase in serovar unspecified is likely due to the introduction of culture-independent diagnostic testing in 2013. Source: NNDSS.

The salmonellosis notification data (all food sources) in Figure 2 is for all non-typhoidal *Salmonella* serovars. The most commonly reported serovars are ST, SE and *Salmonella* Virchow. The relative proportion of specific serovars has become difficult to estimate with the introduction of culture-independent diagnostic methods, where the identity of the serovar cannot be determined. Where serovar information is available, ST is responsible for around 40% of cases, both globally and in Australia.

2.7 Changes in laboratory methods and S. *Typhimurium typing*

A challenge with accurately reporting changes in egg-associated outbreaks since P301 is the change in laboratory methods for sub-typing ST strains that cause foodborne disease outbreaks.

Traditional typing methods used phenotype-based approaches such as phage-typing. *Salmonella* reference laboratories determined the phage type (PT) for ST as well as a small number of *Salmonella* serovars including *S*. Virchow and *S.* Heidelberg. Typing information of ST outbreak strains collected by OzFoodNet up to 2010 include the phage type and, where relevant (e.g. WA-only outbreaks or multi-jurisdictional outbreaks), a genotypic-based method known as pulsed-field gel electrophoresis (PFGE).

In 2011, a new genotypic-based method — multiple loci variable-number tandem repeat analysis (MLVA) — began to be used in many, but not all, Australian jurisdictions. Between 2011 and 2014 OzFoodNet outbreak reports often include both the PT and MLVA profile codes. Victoria reported PT only for ST outbreaks between 2011 and 2014. By 2015, PT began being phased out and MLVA became the only typing information. The last published national OzFoodNet annual report was for 2017, which has limited the amount of national data available[1].

In NSW, ST MLVA typing began to be phased out in 2019 and was replaced with WGS cluster numbering. WA still reported MLVA typing in 2020. Current report sub-typing methods for other jurisdictions is unclear.

Preliminary analysis of the intermediate 2011–2014 period (result not shown) suggests the PT and MLVA profiles were not necessarily consistent within or between jurisdictions. For example, *S*. Typhimurium with the same PT may have a different MLVA profile and similarly, the same MLVA profile may have a different PT. This finding led to more advanced genetic methods using WGS such as core genome multi-locus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) approaches.

Public notification data in the national notifiable disease surveillance system (NNDSS) on *Salmonella* serovars does not include any sub-typing information.

2.8 Egg-associated outbreaks

ST is the main serovar attributed to egg-related outbreaks in Australia. This is in contrast to other countries, such as the UK and USA, where SE is the predominant serovar. Studies have analysed foodborne outbreaks related to *Salmonella* and eggs in Australia for 2001–2016 (Ford et al., 2018; Moffatt et al., 2016). In this period, eggs and egg-containing foods were the most common cause of *Salmonella* outbreaks where a food vehicle could be identified. Eggs were implicated in 30.6% (238/778) of outbreaks (Ford et al., 2018). Food vehicles included eggs, egg-based sauces, desserts containing raw or lightly cooked eggs, and fresh pasta eaten lightly cooked or with a lightly cooked egg-based sauce.

ST was responsible for 95% (226/238) of the egg-related outbreaks, with a large number of other serovars (including Virchow, Saintpaul and Enteritidis) associated with one or two outbreaks each (Ford et al., 2018). Egg-related *Salmonella* outbreaks increased significantly between 2001–2010 (Moffatt et al., 2016), while outbreaks attributed to poultry, beef, pork and other foods trended lower between 2001–2016 (Ford et al., 2018).

Moffatt et al. (2016) identified that 61% (102/166) of egg-related outbreaks in 2001–2010 were prepared by commercial food service businesses (e.g. restaurants and caterers), while 28% occurred in private residence settings. In about 20% of outbreaks (32 of 166), traceback investigations identified the outbreak strain on the farm that produced the eggs[2]. This highlights the difficulty in attributing outbreaks to specific settings, commodities or handling practices at particular points in the supply chain. It also shows there are limitations inherent in traceback investigations, particularly for outbreaks in private residences.

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[1] OzFoodNet annual reports are available at https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-annlrpt-ozfnetar.htm

[2] "Trace back investigations were conducted for 106 (64%) of the 166 outbreaks, with 72 (68%) of these investigations identifying a specific farm from which the implicated eggs had been produced. For these farms, 63 (88%) were inspected and tested. 32 (51%) of the tested farms had phage types or MLV number of tandem repeats analysis patterns detected in the farm environment, on eggs or both, that were indistinguishable from *Salmonella* recovered from outbreak cases." (Moffatt et al., 2016)

Jurisdiction specific OzFoodNet data shows a continuing egg-associated outbreak trend as described by Moffat et al. (2016) and Ford et al. (2018). From 2017-2020 NSW observed 173 enteric disease outbreaks, of which 55 were caused by *Salmonella*. Eggs were associated with 44% (24/55) of these outbreaks. NSW's recently introduced food safety guidelines for the preparation of raw and lightly cooked egg products contributed to a decrease in egg-associated outbreaks (NSW FA, 2023). Within WA, between 2017-2022, there were 153 enteric disease outbreaks, of which 129 were caused by *Salmonella*. Eggs were associated with 42% (55/129) of these outbreaks. For both NSW and WA, identified food vehicles included eggs, egg-based sauces and spreads and desserts containing raw or lightly cooked eggs.

Sodagari et al. (2020) reported that ST was responsible for over 40% of outbreaks in Australia. Other *Salmonella* serovars were also reported to be responsible for small-scale outbreaks (Chousalkar et al., 2018; Kenny et al., 2019; Sodagari et al., 2020). SE is not considered to be endemic in Australian laying flocks. However, the frequency of *Salmonella* outbreaks linked to consuming SE-contaminated eggs suggests there is undetected SE in Australia; and sporadically within Australian commercial laying flocks, the environment and/or wild birds. SE remains a challenge to Australian egg-producers and the current sporadic nature of outbreaks adds to the difficulty in predicting its transmission routes and cost-effective management.

2.9 Recent SE outbreaks linked to eggs

Salmonella Enteritidis (trans-ovarian) was considered absent from Australian layer flocks when Standard 4.2.5 was being developed. However, in 2018–2019 there was a significant multi-jurisdictional outbreak of SE linked to eggs, with 245 people reported ill, and confirmed cases reported in NSW, Victoria, Queensland and Tasmania (NSW FA, 2022). The initial investigation was triggered by an increase in locally acquired SE cases in metropolitan Sydney (Communicable Diseases Branch, 2019). The investigation resulted in a large recall of eggs and a significant biosecurity response involving culling birds and closing egg farms. Following NSW's initial discovery of an SE-positive farm in 2018, a further 16 NSW and five Victorian poultry egg facilities have had SE-positive detections[3]. Investigations identified the movement of people, eggs and equipment between these sites contributed to the spread of SE (NSW FA, 2019). There have also been sporadic notifications of illnesses linked to the outbreak strain since the initial 2018–2019 outbreak investigation was stood down (unpublished).

Notifiable animal disease investigations for SE have continued to be conducted on commercial farms post the 2018-2019 incident. Data collected by Animal Health Australia (AHA) showed 17 positive SE investigations from 2020 onwards (Figure 3). This data is supported by a study by Agriculture Victoria Research, which surveyed Victorian properties after finding a cluster of locally acquired SE cases in 2020 (Muller et al., 2023). They hypothesised this SE cluster was linked to backyard layer hens, either through direct contact or consumption of eggs. These backyard layer hens are often bought from commercial egg farms at the end of their production cycle when they are designated as spent hens. Agriculture Victoria Biosecurity Officers identified 54 properties of interest and sample testing found 8 were positive for SE. Together, these observations from AHA and Agriculture Victoria indicate further investigation into risk factors specific to SE need to be considered in the Australian context.

[3] https://www.dpi.nsw.gov.au/animals-and-livestock/poultry-and-birds/health-disease/salmonella-enteritidis.

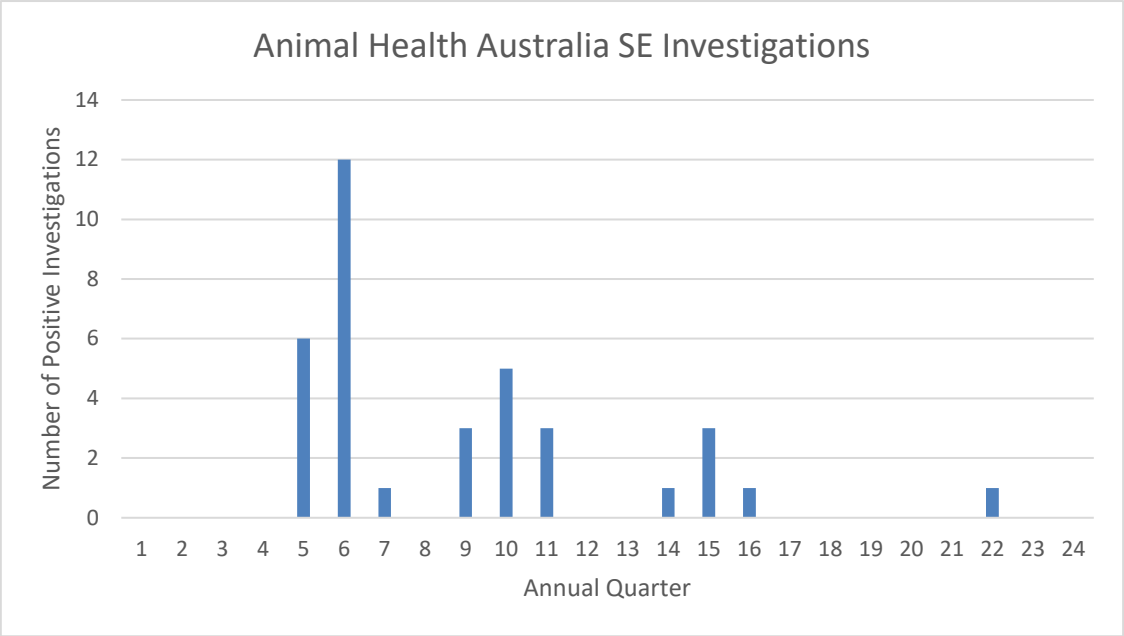

Figure 3: Evidence of on-going farms turning SE-positive – using Animal Health Australia surveillance reporting[4]

Luo et al. (2023) compared 568 SE isolates from NSW and Queensland with 40,390 publicly available genomes from 99 countries. The Australian SE strains were divided into three phylogenetic clades (A, B and C). Clades A and C represented 16.4% and 3.5% of the total isolates, respectively, and were of local origin. Authors also reported the 2018–2019 egg-associated outbreak strain belonged to the clade B lineage, and was closely related (but not directly linked) to European isolates. This study confirms there are endemic strains of SE in the Australian environment, as Clades A and C were not linked to any international clusters. With endemic SE a reality in Australia, the public health risk profile has changed.

2.10 Epidemiological aspects of Salmonella *in laying hens*

Salmonella can infect poultry flocks, with various impacts from causing acute and chronic disease to no obvious symptoms. Infections can be acquired either from parent birds via vertical transmission as a result of reproductive organ colonisation or horizontal transmission from external eggshell contamination (Gast and Porter Jr, 2020; Pande et al., 2016). SE-positive flocks are commonly asymptomatic and young laying flocks may show no obvious symptoms of SE infection. Birds aged >60 weeks may show symptoms of SE infection. During the 2018–2019 SE outbreak, only three (19%) of the SE-infected premises were found to contain poultry exhibiting clinical signs of illness (NSW FA, 2022). These symptoms included depression, increased mortalities associated with peritonitis and an increase in vent pecking and cannibalism. Scott et al. (2020) also reported that during laboratory-controlled exposure of birds to the Australian isolate SE PT7A, no birds demonstrated any overt clinical signs or morphological pathology.

Collins et al. (2023) performed an experimental challenge study with the NSW 2018-2019 outbreak isolate of SE PT12 (isolate No. 760254R1) to demonstrate colonisation and histologic evidence of disease in gastrointestinal and reproductive tracts of commercial layer

[4] Accessed from https://www.sciquest.org.nz/search/results-2/downloadfulltext/173243

hens. During the investigation of the NSW outbreak SE PT12 was found to have caused minimal bird mortalities and clinical signs. However, cloacal excretion of SE and seroconversion were detected in some flocks. Collins et al. (2023) observed SE recovery from caeca, liver, spleen, ovary and oviduct. The study also demonstrated antigenic stimulation and evidence of local infection in multiple organs of point-of-lay hens orally challenged with more than 10^7 CFU/bird SE PT12. Elevated sera IgG antibody titres to Group D *Salmonella* in PT12-challenged birds confirms infection. This result, along with the absence of clinical signs in the challenged birds, agrees with results from similar experimental studies and natural outbreaks involving other invasive SE phage types (Gast and Beard, 1990; Humphrey, 1999; Shivaprasad et al., 1990). While Collins et al. (2023) did not try to determine the source of SE contamination of eggs in their study, they demonstrated SE PT12 can colonise both the gastrointestinal and reproductive tracts of point-of-lay hens challenged with SE.

2.11 Health outcomes for poultry

Salmonella infections can result in varied pathological outcomes in poultry depending on the serovar and age of birds. Importantly, compared to many other serovars, SE has demonstrated significantly higher frequencies of invasion of the caecal lamina and reproductive organs, and more frequent vertical transmission to the internal contents of eggs (Gantois et al., 2008; Gast et al., 2011). However, even within serovars there can be high variation in aspects of infection. For example, SE strains have differed in their ability to cause vertical transmission to the internal contents of eggs from infected hens (Gast and Holt, 2000). These differences have been reported to cross phage-type boundaries, and SE within the same clonal genomic lineage do not have identical virulence characteristics (Olsen et al., 1999). The difference in pathogenicity of SE has been investigated, with Phage Type 4 (PT4) often associated with higher mortality and invasiveness for newly hatched chicks (Gast and Benson, 1995). In contrast, PT4 has been reported to cause similar colonisation, invasive infection, and horizontal transmission in mature hens similar to other phage types (Gast and Benson, 1996; Gast and Holt, 2000).

Salmonella infection can cause markedly different outcomes between younger and more mature birds. *Salmonella* can lead to infection and death in very susceptible young chicks at high rates, with peak levels of mortality being reported for 3–7 days of age (Gast and Porter Jr, 2020). In contrast, older birds are not as susceptible and intestinal colonisation and systematic dissemination may occur without signs of morbidity or mortality (Gast and Porter Jr, 2020). *Salmonella* can also persist internally, with SE recovered from the faeces of 6-month old birds after they were orally inoculated as 1-day-old chicks (Gast and Porter Jr, 2020). In the first weeks following oral infection, adult chickens will typically have high rates of *Salmonella* intestinal colonisation and faecal shedding (Gast et al., 2017). In most cases, this will decline steadily. However, SE has been demonstrated to intestinally persist for several months in adult chickens following oral inoculation. Gut colonisation is normally followed by *Salmonella* invasion via the epithelium and then dissemination and colonisation of internal tissues in older birds.

While some serovars, including ST, are known to be invasive for poultry, research focusing on SE is more frequently documented. Oral inoculation of layer hens has resulted in SE being isolated from various internal tissues, and dissemination to internal organs has followed various inoculation methods including intravenous, intra-tracheal, conjunctival, intravaginal, or intra-cloacal inoculation, contaminated aerosols, or contaminated semen (Gast and Porter Jr, 2020). A dose-dependent relationship has been reported for subsequent levels of internal organ colonisation (Gast et al., 2011). Affected organs have included the liver, ovary and oviduct, which are involved in egg formation.

2.12 Prevalence of Salmonella *in egg contents*

Salmonella positive flocks only produce a small number of contaminated eggs. Studies have found egg contamination may be clustered and intermittent in naturally infected hens (Humphrey et al., 1989).

Directly comparing published results is difficult, with differences in sample sizes due to pooling of eggs and methodologies. For example, surface contamination of eggshells can be tested by swabbing a section of the shell or rinsing the entire shell surface. Egg contents can be sampled aseptically by separating the contents without contact with the shell surface. Alternatively *Salmonella* can be isolated by crushing the egg, allowing contact of the egg contents with shell, and isolating from the mixture.

The prevalence of SE and non-SE *Salmonella* serovars in egg contents is typically low. As a result, it is often difficult to obtain accurate estimates of egg contamination rates without testing enormous numbers of eggs. Schlosser et al. (1999), in a survey of egg contents, detected only 178 (0.028%) SE and 20 (0.003%) non-SE-positive results from 647,000 eggs tested. The median egg prevalence for *Salmonella* in eggs as shown by a quantitative risk assessment model (Thomas et al., 2006) was around one in 20,000 eggs (0.005%) being internally contaminated.

Australian surveys of *Salmonella* in eggs have, however, found surprisingly high prevalence of egg contamination compared to earlier studies. For example, Sodagari et al. (2019) surveyed clean and intact retail eggs in Western Australia in response to the rapid increase in ST cases during 2017 in that state and the epidemiological link to eggs. *Salmonella* was detected in 4.5% (9/200) and 3% (6/200) of egg shells and contents, respectively. *Salmonella* was detected on both shells and in contents for eight samples. *S*. Typhimurium (52%, 12/23) and *S*. Infantis (39%, 9/23) were the most common serovars detected. The cause of the high prevalence is not clear. Reports have also showed that ST can contaminate the internal contents of intact eggs at point of lay, at relatively high prevalence, and can grow within the egg at ambient temperatures (Chousalkar and McWhorter, 2017; Moffatt et al., 2017).

2.13 Survival and growth in eggs

Previous studies of *Salmonella* growth in different egg components have advanced understanding of how SE establishes within eggs and potentially causes illness. Even when SE is initially deposited outside the yolk (on the vitelline membrane or in the albumen), substantial bacterial growth supported by yolk nutrients can occur during the first day of storage (Gast et al., 2010; 2018). The likelihood of bacterial growth increases at higher ambient storage temperatures (Gast et al., 2010, 2018).

ST can penetrate eggshells and survive in the albumin and the yolk at ambient temperatures (Gantois et al., 2008; Gole et al., 2014a). In vitro studies show ST numbers significantly increase in yolk and albumen stored at 25°C, with a higher risk of growth associated with storage at ambient temperatures (Khan et al., 2021). While in vitro models may not directly correspond to naturally contaminated eggs, results indicate growth could occur in commercially produced eggs, with significant public health implications (Gast et al., 2010). Natural contamination of internal contents of eggs by ST has recently been reported in Australia (Crabb et al., 2019; Sodagari et al., 2019), but at a low prevalence and concentration (reported true prevalence of 0.007 (95% CI: 0.001, 0.027), concentration <1 CFU/mL (Crabb et al., 2019).

2.14 Yolk mean time

Infected hens can deposit *Salmonella* in either the yolk or albumen of developing eggs because of the colonisation of different regions of the reproductive tract. However, Gast et al. (2008) reported that the nutrient-rich yolk interior is an uncommon location for SE contamination in freshly laid, naturally contaminated eggs. *Salmonella* deposited in the albumen or on the outside of the vitelline membrane need to survive and grow in this antibacterial environment, before they can migrate to and penetrate the membrane to reach the nutrient-rich yolk. The time to break down the vitelline membrane is sometimes referred to as the yolk mean time (YMT). At YMT *Salmonella* present in the egg should be able to begin exponential growth (Thomas et al., 2006; Whiting et al., 2000).

Studies have shown the egg can resist SE growth for around 2 to 3 weeks at room temperature (Humphrey et al., 1991). Under elevated temperatures, access to the yolk may become easier over time, as the albumen viscosity and vitelline membrane integrity decline (Hara-Kudo et al., 2001; Humphrey and Whitehead, 1993; Messens et al., 2004). After the loss of membrane integrity, the survival of *Salmonella* in albumen and growth on reaching the yolk are temperature- and serotype-dependent (Gast et al., 2007).

Chen et al. (2005) reported on the outgrowth of SE and the physical properties of albumen and vitelline membranes as influenced by egg storage conditions. Storage at 4°C was reported to preserve the antimicrobial agents of the albumen and maintain the integrity of vitelline membranes. Growth of a mix of five SE strains was inhibited at 4°C for 6 weeks, after inoculation into the albumen at initial populations of 10^2, 10^4 and 10^6 CFU/egg. In comparison, egg storage at 22°C led to significant deterioration of the vitelline membrane and SE was reported to flourish, even in the albumen with the lowest initial population (10^2 CFU/egg). The main biochemical change during egg aging responsible for the loss of the membrane's integrity is the degradation of glycoprotein II. Some experimental data showed eggs held at room temperature (25ºC) for a week lost 75% of the membrane strength because of glycoprotein degradation (Kirunda & McKee, 2000). Similarly, Clay and Board (1991) reported SE growth occurred in the albumen of eggs stored at 25°C but not at 4°C. The storage of eggs at refrigeration temperatures is an effective way of reducing the liquefaction of egg white, the loss of integrity of the vitelline membrane, and consequently, bacterial penetration and growth. However, while chill storage may inhibit growth, viable cells may still be present. Therefore, for SE growth to be effectively reduced, eggs would need to be refrigerated from shortly after lay and through to cooking and consumption.

Whiting et al. (2000) developed a YMT model to estimate SE growth during egg collection, processing, storage and transportation. The input values (e.g. for time, temperature, cooling rate) and distributions represented estimates of industry practices in the USA. The model contained equations for the internal egg temperature, yolk membrane integrity and exponential growth rate of SE. This model also assumed no growth of *Salmonella* will occur until after the YMT had been reached. Following the development of the original YMT model, significant variations in the ability of *Salmonella* serovars to penetrate the vitelline membrane have been observed (Cogan et al., 2004). Conditions to support growth at a given temperature in an individual egg may vary substantially and because of this, large confidence intervals are associated with YMT predictions. Taken together, the YMT model may not reflect the behaviour of all *Salmonella* serovars in eggs and may result in a conservative risk estimate (Thomas et al., 2006).

3 HAZARD CHARACTERISATION

Summary of hazard characterisation

Salmonella produces endotoxins, enterotoxins, and cytotoxins, contributing to its pathogenicity in poultry. Endotoxins induce fever, enterotoxins cause fluid accumulation, and cytotoxins damage epithelial cells. *Salmonella* adheres to intestinal epithelial cells via flagella and fimbria, facilitating invasion and disease. Lipopolysaccharide (LPS) and virulence genes on *Salmonella* pathogenicity Island 1 (SP-1) also play roles in invasion. Pathogenicity is associated with serovar-specific plasmids carrying virulence and antibiotic resistance genes. These plasmids enhance survival within macrophages and in serum.

Studies from the 1950s on healthy males showed a relationship between ingested dose and infection, but they may underestimate pathogenicity for the general population. These trials used high doses, whereas real food contamination involves much lower doses. Fazil (1996) combined all the data from the feeding trials and found that a single beta-Poisson relationship could adequately describe the dose-response for all serovars. It was generally assumed that it takes a dose of at least 10^7–10^9 cells to cause salmonellosis. However, data from outbreaks of salmonellosis have indicated that sometimes doses even below 10^3 cells are able to cause gastroenteritis. The WHO/FAO developed a dose-response model based on outbreak data, which is more reflective of real-world conditions. Thomas et al. (2006) re-evaluated this data for the Australian quantitative risk assessment model. In FSANZ's current risk assessment model, we use a beta-Poisson dose-response model adapted from Thomas et al., (2006).

Individual susceptibility varies with factors like immunity, nutrition, age, and pre-existing conditions. Vulnerable groups include the very young, elderly, pregnant women, and immunocompromised individuals (e.g. organ transplant recipients, cancer patients, AIDS patients).

3.1 Virulence and infectivity

Toxins are important for the pathogenicity of salmonellae in poultry. Endotoxins associated with the cell wall of *Salmonella* can induce fever if released into the blood stream of infected chickens (Gast and Porter Jr, 2020). Enterotoxins result in epithelial cell responses of excretion and fluid accumulation in the lumen (Gast and Porter Jr, 2020). Cytotoxins are known to cause structural damage to epithelial cells (Gast and Porter Jr, 2020).

As with humans, adherence and invasion are required for salmonellae to cause disease in poultry (Gast and Porter Jr, 2020). Adherence to intestinal epithelial cells is facilitated by attachment mediated by flagella and fimbria (Dibb-Fuller and Woodward, 2000). LPS is also proposed to be involved in attachment (Carroll et al., 2004). Expression of virulence genes on *Salmonella* SP-1 is reported to facilitate invasion of internal tissues in chickens, but is less involved with caecal colonisation (Desin et al., 2009). Flagella have been demonstrated to have a role in the invasion and subsequent dissemination of SE into the internal organs of chicks (Gast and Porter Jr, 2020). Type 1 fimbria may mediate colonisation of the upper oviduct (De Buck et al., 2004).

Plasmids can be associated with pathogenicity and serovar-specific plasmids have been linked with virulence of salmonellae (Gast and Porter Jr, 2020). Genes promoting survival within macrophages are prevalent among *Salmonella* virulence plasmids (Rychlik et al., 2006). ST and SE plasmid-mediated virulence has been associated with survival and multiplication in serum. *Salmonella* without virulence-associated plasmids have

demonstrated reduced persistence in the caeca of chicks (Virlogeux-Payant et al., 2003). However, SE that had a serovar-specific plasmid removed did not result in decreased invasion of intestinal tissues in chickens (Chart et al., 1996). Plasmids carrying virulence genes may also carry antibiotic resistance genes and facilitate conjugative transfer (Han et al., 2012).

3.2 Dose response

Human feeding trials for a range of *Salmonella* serovars were done in the 1950s to determine the relationship between the dose of pathogen ingested and the response of the individual (McCullough and Eisele, 1951a, 1951b, 1951c, 1951d). The study population consisted of healthy males confined in an institutional setting who were fed known doses of an individual *Salmonella* serovar. Infection was confirmed by recovering the administered *Salmonella* serovar from faecal samples.

Fazil (1996) combined all data from feeding trials and found a single beta-Poisson relationship could adequately describe the dose-response for all serovars. However, there are limitations on using such feeding trial data. Firstly, relying only on healthy adult male volunteers could underestimate the pathogenicity to the overall population. In addition, volunteers were exposed to high doses of *Salmonella*, with the minimum dose being 10^4 cells.

In dose-response analysis, the critical region is the lower-dose region, as these are the doses most likely to exist in real food contamination events. This requires extrapolation of the model to doses much lower than those used in human feeding trials. Also, the dose-response models are based on the risk of infection as an endpoint rather than illness, and therefore may introduce a level of conservatism into the dose-response relationship.

It was generally assumed that it takes a dose of at least 10^7–10^9 cells to cause salmonellosis. However, data from outbreaks of salmonellosis have indicated sometimes doses even below 10^3 cells are able to cause gastroenteritis. Using a reasonably large data set, the WHO/FAO in 2002 developed a dose-response model based on actual outbreak data. Again, a beta-Poisson model was used to describe the dose-response relationship (Figure 4; Thomas et al., 2006).

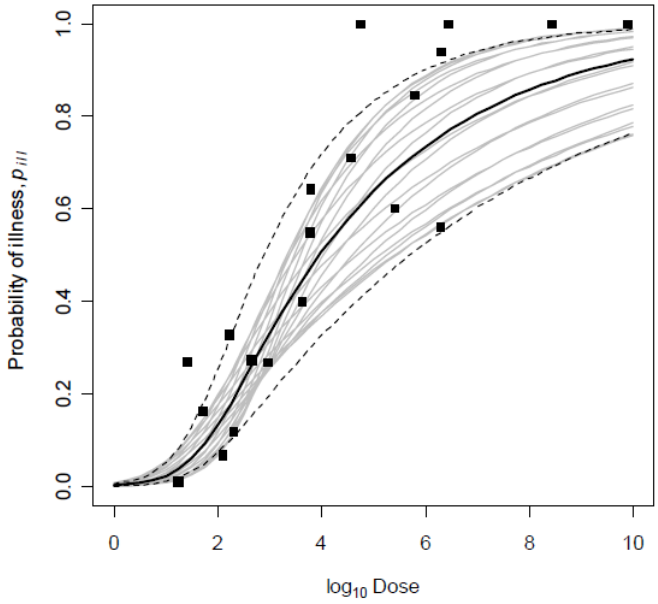

Figure 4: Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002; Thomas et al., 2006).

Further discussion on the data used to generate the dose response model can be found in FAO/WHO (2002).

An Australian quantitative risk assessment model for *Salmonella* in eggs was developed by Thomas et al. (2006). They re-evaluated the WHO/FAO outbreak data and estimated the values for α and β (Table 1) and used an alternative beta-Poisson dose-response equation (Equation 1). At low doses (<100 cells) and at high doses (>10^6 cells), the predicted probability of illness was similar between the WHO/FAO model and the alternative dose-response model. At intermediate doses, estimated probabilities of illness from the alternative dose-response model were less variable than the WHO/FAO model (Thomas et al., 2006). In this risk assessment model, we utilized a beta-Poisson dose-response model adapted from Thomas et al. (2006).

Equation 1

$$p_{ill} = 1 + \left(\frac{Dose}{10^{(\log_{10} \beta)}} \right)^{-10^{\log_{10} \alpha}}$$

Table 1 Predicted $\log_{10} \alpha$ and $\log_{10} \beta$ values for the dose-response model (modified from Thomas *et al*., 2006).

Parameter	Distribution	Expected Value
$\log_{10}\alpha$	Normal	-0.8729744
$\log_{10}\beta$	Normal	1.725438

Variance/Covariance Matrix

$$\begin{pmatrix} 0.008035438 & 0.01801451 \\ 0.018014510 & 0.05149408 \end{pmatrix}$$

3.3 Host factors

Individual susceptibility to *Salmonella* infection and/or disease can vary significantly, depending on host factors such as pre-existing immunity, nutrition, age, ability to elicit an immune response, structural and functional anomalies of the intestinal tract, or pre-existing disease (Gerba et al., 1996; Jay et al., 2003). Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Salmonella* include the very young, the elderly, pregnant women and the immunocompromised (organ transplant patients, cancer patients, AIDS patients) (Gerba et al., 1996).

4 EXPOSURE ASSESSMENT

Summary of exposure assessment

Eggs are formed in the ovary and the liver of a hen, with components produced sequentially. The stages include yolk deposition, albumen production, shell membrane formation, and shell formation, taking approximately 26 hours. The egg has several barriers to microbial invasion, including the cuticle, shell, shell membranes, and vitelline membrane. The albumen contains antimicrobial compounds like ovotransferrin and lysozyme. The pH of the albumen increases over time, enhancing these antimicrobial properties.

SE can colonise the oviduct and survive in egg white, leading to internal contamination of eggs. Vertical transmission is common, often resulting in low contamination levels (<10–20 CFU/egg). ST has variable results in vertical transmission studies and is less significant in internal contamination of eggs. Horizontal infection via contaminated faeces is the main route for ST contamination during lay. Certain genes are linked to SE's ability to infect reproductive tissues and survive in eggs, including those involved in stress responses and cell wall biosynthesis. Eggs can be externally contaminated via contact with the chicken's vent, faeces, or contaminated environments. Factors like faecal contamination, time, temperature, and moisture influence the ability of *Salmonella* to penetrate the eggshell.

To multiply within an egg, SE must gain access to nutrients from the yolk. This happens over time as the yolk membrane begins to degenerate and become permeable. The membrane degrades faster at higher storage temperatures.

The risk of *Salmonella* infection is influenced by flock age, housing facilities, and multiple-age stocking. Persistent environmental contamination in egg production facilities can spread infections. *Salmonella* is often distributed in association with dust and faeces and can be perpetuated by rodent or insect infestations, which can survive standard cleaning methods.

Effective biosecurity practices reduce the risk of *Salmonella* contamination. These include controlling access to premises, handwashing, rodent and insect control, and maintaining feed and water quality. Practices such as equipment sharing between sheds and limited equipment disinfection are examples of poor biosecurity activities.

Effective sampling programs are crucial for detecting *Salmonella* in poultry flocks. Environmental sampling, including of faeces and dust, is key to identifying SE in laying houses. Methods include cloacal swabs, gauze swabs, shoe covers, and collecting litter or dust. Traditional culturing methods are used for *Salmonella* detection, with whole genome sequencing (WGS) recommended for identifying genetic variations and sources of infection.

Salmonella contamination and its spread are commonly mitigated through a variety of techniques, including egg washing, vaccination, refrigeration and maintaining egg traceability. Commercial egg washing involves multiple stages to reduce microbial load. Regulations vary, with some countries prohibiting washing to avoid cuticle damage (refer to SD 3). Proper washing techniques are essential to prevent bacterial ingress into the egg. Vaccination, combined with good sanitation and biosecurity, helps manage *Salmonella*. Both live attenuated and inactivated vaccines are used, with live vaccines generally more effective. Vaccination alone cannot guarantee *Salmonella*-free flocks without good hygiene practices. Refrigeration of eggs post-lay minimises *Salmonella* growth. Regulations differ, with some countries requiring refrigeration and others avoiding it to prevent condensation. Robust traceability systems are essential for managing food safety incidents. In Australia, eggs must be marked with a unique identifier for traceability. Enhanced traceability measures are recommended to limit the extent of *Salmonella* outbreaks.

4.1 Egg formation and characteristics

A comprehensive review[5] of egg formation and chemistry is provided by Nys and Guyot (2011). Egg components are produced sequentially by two different anatomical structures; the liver and the ovary. Egg yolk components are first produced in the liver and are then deposited in the ovary via the bloodstream. Figure 5 summarises sequential formation of the egg in the genital tract of hens. The ovulation of the yolk then occurs in the ovary. The yolk is captured by the infundibulum where it remains for approximately 15 minutes (Nys and Guyot, 2011; Roberts, 2004). It is at this stage the vitelline membrane and chalazae are formed. The egg then passes through the magnum where it remains for 2.5–4 hours while the albumen is produced (Board et al., 1994; Nys and Guyot, 2011; Roberts, 2004). Following this, the egg moves through the isthmus, where shell membranes are produced (taking approximately 1 hour) (Nys and Guyot, 2011). The egg then enters the tubular shell gland where water and electrolytes enter the albumen (termed “plumping”). The egg spends approximately 15–20 hours in the uterus where the eggshell is formed and the process of “plumping” is completed (Nys and Guyot, 2011). Once completed, the egg is laid via the vagina, cloaca and vent. Overall, the time required for the formation of an egg is approximately 26 hours (Keller et al., 1995; Nys and Guyot, 2011).

Eggs have a complex series of physical and chemical barriers to microbiological invasion and growth (Nys and Guyot, 2011). While maintaining integrity from bacterial invasion, eggs possess about 10,000 pores for exchange of respiratory gases and water vapour during growth of the embryo (Nys and Guyot, 2011). However, these pores also present a potential route for microorganisms to penetrate the egg (Bruce and Drysdale, 1994).

The initial physical barrier to microbial penetration of the egg is a fine hydrophobic proteinaceous layer called the cuticle (Pertinez et al., 2020). The cuticle covers the egg which, when dry, forms “plugs” within the pores providing enhanced protection from microbial penetration (Kulshreshtha et al., 2018).

In addition to the external barriers of the shell and cuticle, inner shell membranes (separating the internal surface of the shell and albumen) and the vitelline membrane (separating the albumen and yolk) provide further barriers to microbial penetration (Nys and Guyot, 2011). In addition to providing a physical barrier to microbial invasion, these semipermeable membranes are involved in diffusion of gases to and from egg compartments (Bruce and Drysdale, 1994).

Albumen contains a number of compounds inhibitory to bacterial survival and/or growth. Approximately 12–13% of albumin consists of ovotransferrin which chelates metal ions required for microorganisms to grow, and 3–4% lysozyme which can lyse bacterial cells (Baron and Jan, 2011). Freshly laid eggs have a pH in the range of 7.6–7.8; however, after 1 to 3 days storage at room temperature, pH rises to 9.1–9.6, at which ovotransferrin has an enhanced ability to chelate metal ions (ICMSF, 1998; Li-Chan et al., 1995). In contrast, egg yolk provides an ideal medium for bacterial growth (Board et al., 1994). The pH of the yolk at time of lay is approximately 6.0 and can increase to 6.9 during storage (Li-Chan et al., 1995).

[5] see also https://www.youtube.com/watch?v=G6j13-9Pexw

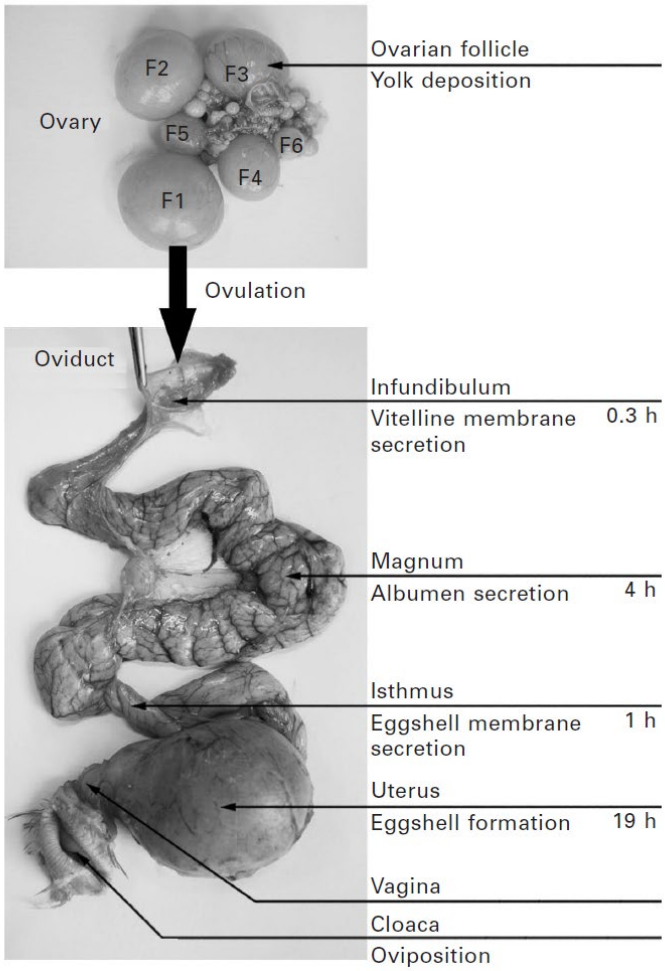

Figure 5: Sequential formation of the egg in the genital tract of hens. Photographs represent hen ovary (top) and oviduct (bottom). Phases of egg formation and their approximate durations (in hours) are indicated below each associated site. Ovarian follicles (identified F1 to F6) in the ovary are the pre-ovulatory follicles of the ovarian hierarchy (Nys and Guyot, 2011).

4.2 Contamination of eggs during formation

Research on the potential of ST to transmit vertically have produced variable results. ST has not been regarded as a significant serovar in terms of its ability to internally contaminate eggs during formation and survive until point of lay (Gantois et al., 2008; Pande et al. 2016). ST has been reported to invade reproductive organs (Gantois et al., 2008; Keller et al., 1997; Okamura et al., 2010), follicular tissues, and the forming eggs of layer hens (Okamura et al. 2001a). Oral or feed infection with ST has not been associated with subsequent internal contamination of eggs (Gantois et al., 2008; Keller et al., 1997; Okamura et al., 2001a; Pande et al., 2016). However, inoculation of pullets at the beginning of their production cycle (16 weeks) orally (Okamura et al., 2010), by aerosol inoculation (Leach et al., 1999), and by intravenous inoculation (Okamura et al., 2001b) have been reported to result in ST internally contaminating eggs.

ST can enter a viable but non-culturable state on exposure to egg white (Passerat et al., 2009). Differentiating this state from inhibitory albumen effects is challenging (Carrique-Mas and Davies, 2008; Chousalkar et al., 2018). The reduced ability of ST to transmit vertically may be due to limited capacity to survive and proliferate in egg contents during egg formation

at the host body temperature (42°C) (Pande et al., 2016). Alternatively, it could be due to down-regulation of genes critical to colonisation (Pande et al., 2016). A recent study on the long-term shedding, egg contamination and oviduct colonisation by ST concluded horizontal infection through contaminated faeces is the main route of ST contamination during lay (Pande et al., 2016).

Vertical transmission of SE occurs within the reproductive tract of infected hens before the shell forms. This is largely due to the ability of the bacterium to persist and multiply long term, possibly for the whole of the life of an individual hen, in the ovary or glandular tissue of the oviduct (Berchieri et al., 2001). Deposition of *Salmonella* during the formation of eggs typically involves very small numbers of bacteria (<10–20 CFU/egg) and is likely to preferentially occur within the albumen or yolk membrane. Egg contamination seldom occurs at high frequencies, and does not usually involve large numbers of bacteria, even when hens are infected with massive oral doses of SE (Gast and Holt, 2000; Gast et al., 2019). Experimentally infected hens laid eggs with an SE contamination incidence of 2.5% of yolk and 0.5% of albumen samples (Gast and Holt, 2000). Most of the contaminated eggs contained fewer than one SE cell/ml of egg yolk or albumen, and no sample contained more than 67 SE cells/ml (Gast and Holt, 2000).

Direct contamination of the yolk is rare, but if it happens, *Salmonella* can multiply in the yolk's nutrient-rich environment. It has been hypothesised SE employs a stress-induced survival mechanism to colonise the oviduct and subsequently survive in the egg white (Van Immerseel, 2010). If this was a common route for egg content contamination, newly-laid, naturally-infected eggs would be found with high numbers of SE in them. However, this is a rare event (Humphrey, 1994). Multiplication of SE deposited in albumen or membranes is restricted by multiple inhibitory factors in the albumen and the scarcity of iron (Baron et al., 2016).

If SE has infected the oviduct, bacteria can be deposited into external layers of albumen, shell membranes or onto the shell – this depends on the section of the organ that is colonised and the timing of shedding of bacteria into eggs (De Vylder et al*.,* 2013). Forming eggs produced by infected hens have a much higher contamination rate than eggs after laying, suggesting in many cases the low level of contamination introduced during egg formation does not survive (Keller et al., 1995). To multiply, SE must gain access to nutrients from the yolk, which happens over time as the yolk membrane begins to degenerate and becomes permeable. The membrane degrades faster at higher storage temperatures (Gross et al., 2015). Degradation may be followed by diffusion of yolk into the albumen and chemotaxis of SE towards the yolk (Gross et al., 2015).

Several genetic factors are involved in the infection of hen reproductive tissues and in resistance to the inhibitory effect of egg albumen. Enhanced invasion of the reproductive tract and survival of SE and *S*. Gallinarum in the forming egg has been linked to the presence of SEF-14 fimbriae (Peralta et al., 1994; Thiagarajan et al., 1996; Rajashekara et al., 2000; Rank et al., 2009). *In vivo* gene expression technology revealed SE universal stress protein genes *uspA* and *uspB* were highly expressed in the chicken oviduct and eggs. Mutations in these genes compromised the ability to infect reproductive tissues and forming eggs (Raspoet et al., 2014).

Shah et al. (2012) examined the role of SE virulence genes in the infection of human gut epithelial cell lines, chicken liver cells and macrophages using transposon mutagenesis. Many genes were found to be important and some of these appeared to be almost specific to SE. Raspoet et al. (2014) used microarray detection to identify genes important in survival of SE in primary chicken oviduct gland cells *in vitro* and persistence in the reproductive tract *in vivo*. Eighty genes were found to be important and major groups included those involved in

stress responses and cell wall and LPS biosynthesis. Coward et al. (2013) found expression of very long LPS O antigen in SE is essential for egg contamination, probably linked with better infection of the reproductive tract and survival of the bacteria in egg albumen *in vivo*. In earlier work, Coward et al. (2012) examined five pathogenicity islands in SE, namely R1, 3, 4, 5 and R6. All played a small role in the infection of liver and/or spleen but not in the infection of the reproductive tract.

Van Immerseel (2010) found stress-induced bacterial survival strategies are important in allowing SE to persist in hen reproductive tracts. McKelvey et al. (2014) demonstrated SE antimicrobial peptide resistance genes are important in colonisation of the intestine and infection of the reproductive tract. Related artificial infection studies showed the levels of SE used to infect birds influenced egg contamination patterns seen. Higher oral doses of SE PT4 resulted in greater contamination of egg contents, with albumen being more likely to be SE-positive than yolk (Gast et al., 2013)

Host factors are also important in egg contamination processes. Loss of protective immunity systemically and in the reproductive tract when birds reach sexual maturity, increases susceptibility to SE, even in vaccinated hens (Johnston et al., 2012).

4.3 Contamination of the eggs from point of lay

When the egg is laid, the shell may be externally contaminated by microorganisms and, because the shell is porous, internal penetration can occur (Gantois et al., 2009a; Ray et al., 2015). *Salmonella* may be transmitted by contact with the bird's vent during laying or through contact with faeces or other sources in the surrounding environment (e.g. faeces-contaminated nest boxes, conveyer belts, floor) (Gantois et al., 2009a).

The level of faecal contamination (Schoeni et al., 1995), time and temperature (Gantois et al., 2009b; Gast et al., 2005; 2008; Whiley et al., 2016), moisture (Gantois et al., 2009a; Gradl et al., 2017; Padron, 1990; De Reu et al., 2006), intrinsic characteristics of the egg (Chousalkar et al., 2010; Gantois et al., 2009a; Gole et al., 2014a; 2014b; Messens et al., 2007; Pinto and Silva, 2009; Ray et al., 2015), and characteristics of the serovar (Gantois et al., 2009a) all potentially impact *Salmonella'*s ability to penetrate the shell and its contents. Both SE and ST, as well as other serovars, can penetrate egg shells and contaminate the internal contents of eggs after laying.

4.4 On-farm risk factors

Commercial egg production facilities are highly complex environments. It is unclear whether or how different production systems impact *Salmonella* infection rates. The introduction and spread of *Salmonella* in laying flocks both largely depend on environmental conditions in egg production facilities (Trampel et al., 2014).

Commonly identified risk factors linked to increased *Salmonella* infection in laying hens are flock age, housing facilities, and multiple-age stocking (Mollenhorst et al., 2005; Namata et al., 2008; Huneau-Salaün et al., 2009; Snow et al., 2010; Van Hoorebeke et al., 2010; Pitesky et al., 2013; Denagamage et al., 2015). The poultry house environment often serves as a reservoir for SE. Persistent environmental contamination can sometimes cause transmission of infection into successive laying flocks over extended periods (Davies and Breslin, 2003; Dewaele et al., 2012a, 2012b; Lapuz et al., 2012).

Salmonella contamination is often widely distributed throughout laying houses in dust and faeces (Garber et al., 2003; Kinde et al., 2005; Im et al., 2015). Contamination can be perpetuated and amplified by severe rodent or insect infestations, reaching levels that

survive standard cleaning and disinfection (Carrique-Mas et al., 2009; Snow et al., 2010; Lapuz et al., 2012; Wallner-Pendleton et al., 2014).

4.5 Biosecurity

Once introduced into laying houses, *Salmonella* can spread rapidly and extensively throughout flocks by direct contact between hens, ingestion of contaminated feed or faeces, movement of personnel and equipment, and airborne circulation of contaminated dust and aerosols (Gast et al., 1998; 2014b; Thomas et al., 2009; 2011). Environmental stressors including feed deprivation, water deprivation, or excessive heat, can increase hens' susceptibility to horizontally transmitted infections (Asakura et al., 2001; Humphrey, 2006; Okamura et al., 2010).

Effective biosecurity practices are critical in integrated programs aiming to reduce the risk of SE and other *Salmonella* serovars on an egg farm. These practices reduce the probability of pathogens entering an egg-production facility. They include control of persons and equipment entering premises, handwashing, disposal of dead birds, vermin control, record keeping, wildlife and pest control, and feed and water quality. Proper implementation of biosecurity protocols maintains good poultry health and welfare on farms. It also reduces financial losses by decreasing the frequency and magnitude of infectious disease outbreaks (Scott et al., 2018).

There are reports of low adoption of biosecurity practices in different egg production systems in Australia (Scott et al., 2018). For instance, inadequate distance between sheds, and between sheds and waterbodies; equipment frequently shared between sheds and not disinfected; footbaths and visitor recording books infrequently used. Additionally, potential infection via wild birds also need to be considered, particularly for free-range farms. Furthermore, dog and cat access to layer farms and the range environment present breaches of necessary biosecurity practices.

4.5.1 Housing

International studies report conflicting data on *Salmonella* prevalence across different housing systems. In Europe, environmental testing of layer flocks showed a higher prevalence of *Salmonella* in flocks housed in conventional cages compared with those housed on the floor. This was observed in multiple countries (Germany: Methner et al., 2006; United Kingdom: Wales et al., 2007; Snow et al., 2010; France: Mahé et al., 2008; Belgium: Namata et al., 2008). A Danish study found human SE disease was associated with eggs from conventional cages but not from free-range or organic operations (Mølbak and Neimann, 2002). Conversely, other studies detected a lower incidence of *Salmonella* in conventional cage systems than cage-free systems (United States: Kinde et al., 1996; Germany: Schaar et al., 1997; Netherlands: Mollenhorst et al., 2005). A Swedish study found that there was no difference in the exposure of flocks to *Salmonella* between outdoor and indoor production setups (Wierup et al., 2017). A survey by the USDA Animal and Plant Health Inspection Service found pullets raised in conventional cages had lower SE incidence than floor-raised pullets (USDA, 2000). Reasons for the disparity in results are unknown. Three of the four studies showing higher incidence in conventional caged layers were conducted on flocks within 9 weeks of end of lay (Methner et al., 2006; Mahé et al., 2008; Snow et al., 2010). *Salmonella* incidence tends to increase with flock age (Wales et al., 2007). The higher incidence in conventional cage facilities may be a reflection of sampling logistics, and faeces and resident *Salmonella* localised in manure pits beneath the cages (rather than being spread over a wide area in floor-raised facilities).

4.5.2 Sharing equipment

Scott et al. (2018) found equipment was shared between sheds in 78% of cage layer farms, 78% of barn layer farms and 92% of free-range layer farms. Shared equipment was not disinfected on the cage layer farms, but was disinfected in 14% of the barn layer farms and 9% of the free-range layer farms (Scott et al., 2018). The NSW investigation of the 2018–2019 SE outbreak in egg layer farms highlighted the complex movement of people, vehicles and equipment between premises. Failure to maintain stringent biosecurity practices was a leading factor in the spread of SE between facilities (NSWFA, 2022).

4.5.3 Rodents

Mice are known to spread and amplify SE in chicken houses and are a primary reservoir and source of infection for laying hens (Trampel et al., 2014). Mice find chicken houses ideal places to live because food, water, and shelter are readily available. SE infection of successive chicken flocks has been linked to the presence of rodents and low decontamination standards. Mice and rats are mobile and can spread *Salmonella* from one flock to another, to adjacent houses on the same premises, and to nearby farms.

Mice are more susceptible to SE than most other paratyphoid *Salmonella*, with only 15 SE cells required to infect a mouse (Trampel et al., 2014). Theoretically, one contaminated mouse could deposit up to 23 million SE cells in a chicken house in a single day.

Effective control programs should be in place to keep rodent numbers in chicken houses as low as possible. Measures should include (1) repairing holes that allow rodent entry, such as worn door seals and holes near the house foundation; (2) removing vegetation and debris from the outer perimeter to eliminate harbourage sites; (3) selecting effective baits and bait placement; (4) promptly and securely disposing of any dead birds or unused or spilled feed; and (5) regularly repeating rodent inspections, baiting and trapping. Rodent numbers should be monitored by visual inspection and use of mechanical traps (Trampel et al., 2014).

4.5.4 Insects

Control of insect vectors is also critical in reducing the risk of SE on poultry farms. SE has been isolated from flies in contaminated laying hen houses, and chickens ingesting contaminated flies have become infected (Trampel et al., 2014). Contaminated flies, beetles, and other insects may serve as SE reservoirs and allow SE to survive in an egg facility, even after cleaning and disinfection.

4.5.5 Wild birds

Control measures to discourage wild and migratory birds on egg production sites should be in place. SE has been isolated from wild bird droppings collected from contaminated chicken farms (Trampel et al., 2014). Buildings should be constructed to exclude wild birds, so they cannot nest and reproduce in chicken houses. Trees or branches overhanging the chicken house roof should be removed to discourage perching, nesting and deposition of droppings on the roof. Any feed spills outside buildings should be cleaned up immediately to avoid attracting wild birds and rodents.

4.5.6 Feed

Even though feed is not a frequent route of transmission, SE has been isolated from finished feed and feed ingredients (Trampel et al., 2014).

4.5.7 Spent hens

Spent hens are commercial egg-laying hens no longer considered economically viable for commercial egg production. These hens are mainly culled and buried on-site, or transported to a processing facility to make fertiliser, pet food or low quality meat for human consumption. The use of spent hens as backyard poultry egg-layers has been reported in Australia and other countries (Graham et al., 2021).

In Australia, the National Farm Biosecurity Technical Manual for Egg Production (clause 4.4.2), advises “A record of bird movements must be maintained to facilitate tracing in case of an animal health or food safety concern” (AHA, 2023). However, there is a lack of information on (i) the SE testing requirement prior to bird movement, (ii) the numbers and frequencies of spent hens being sold for backyard egg production, and (iii) their input to the Australian egg industry. Due to their age, spent hens are more susceptible to SE infection and the impact of infection presents a higher risk for egg contamination.

The prevalence of *Salmonella* in spent hens in Australia is not well known. Overseas studies indicate a variable but potentially high prevalence (7–61%) of *Salmonella*, including SE.

A study from China reported 19% prevalence of *Salmonella* spp. in spent hens sold for meat in a food market. Among the serotypes detected, SE was the most prevalent (52.6%), followed by ST (31.6%) and *S.* Derby (15.8%) (Li et al., 2017). A US survey of 23,431 pooled caecal samples from 10 spent-hen processing plants covering 406 layer houses indicated high (24%) *Salmonella* prevalence, including 3% SE (Ebel et al., 1992).

In a Japanese study of 16,000 spent hens from 23 farms, hens from 52.2% farms were positive for 23 serotypes of *Salmonella,* including SE. The prevalence rates in the hens' caeca, immature eggs, and the yolk of mature eggs in oviducts were 14%, 7.2%, and 6.8%, respectively (Otomo et al., 2007). In the Netherlands, a *Salmonella* prevalence rate of 61.4% in spent hens was reported. Prevalence was higher in older hens (≥80 weeks) than younger hens (≤60 weeks) (De Reu et al., 2006).

Salmonella illness outbreaks are frequently reported associated with backyard hens. In 2020, the US Centers for Disease Control and Prevention (CDC), reported a multistate outbreak of *Salmonella* infections linked to backyard poultry, resulting in 1,722 illnesses, 333 (33%) hospitalisations and one death (Nichols et al., 2021). Australia has seen similar SE outbreaks associated with backyard hens. For example, in 2020 the Victorian Health Department urged owners of backyard chickens to observe safe handling practices, following a surge in SE infections (McNaughton, 2020). Agriculture Victoria confirmed SE was detected at a commercial egg farm and likely chickens from that farm were sold to at least one of the affected backyard flocks (McNaughton, 2020).

4.6 Environmental monitoring

Efficiency of sampling programs has a big impact on detecting *Salmonella* and estimating prevalence (Fletcher, 2006). Thorough environmental sampling is the most effective way to detect zoonotic serovars of *Salmonella* in poultry (Aho, 1992; Johansson et al., 1996; Musgrove and Jones, 2005). Identification of SE in laying house surroundings is linked to contaminated eggs from infected flocks. Environmental sampling is commonly used for initial screening to identify possibly infected flocks that require further investigation. This is an effective approach, given the low frequency of contaminated eggs produced by such flocks (Gast, 2007; Trampel et al., 2014).

Faecal shedding by infected hens is a major contributor to *Salmonella* contamination in poultry houses. However, evaluation of faecal samples alone does not always predict infection rate in a farm (Wales et al., 2006). Faecal shedding of SE appears to be associated with stress and peaks just before egg laying commences in commercial flocks (Li et al., 2007; Gole et al., 2014c).

Cloacal swabs from individual birds can indicate the prevalence of SE infection within flocks. However, swabs are relatively insensitive compared to other detection options and swabbing has animal welfare impacts (García et al., 2011; Schulz et al., 2011).

Dust samples may provide more sensitive SE-positive results than faecal and other environmental samples (Gole et al., 2014c; Arnold et al., 2010; Martelli et al., 2014). Testing both dust and faeces on a layer farm appears to be particularly effective in an environmental monitoring scheme. This approach helps compensate for variations in detection in either sample (Carrique-Mas and Davies, 2008; Arnold et al., 2010).

There are several common methods for collecting environmental samples in poultry farms. These include dragging gauze swab assemblies across floor surfaces, walking through houses wearing absorbent fabric shoe covers, and collecting litter material or dust from locations such as egg belts, fan blades, or nest boxes (Lungu et al., 2012; Davies and Breslin, 2001).

Salmonella is usually isolated and identified from environmental samples using traditional selective enrichment culturing methods, followed by biochemical and serological confirmation. Rapid assays (based on the recognition of specific genetic sequences or antigenic molecules) are becoming increasingly popular (Waltman and Gast, 2008). However, these detection methods do not identify genetic variation or the source of infection. WGS should be considered for *Salmonella*-positive samples to identify the genetic variation, virulence and source of infection.

4.7 Mitigation strategies

4.7.1 Washing eggs

Commercial washing of eggs involves four general stages: (i) a pre-wash or wetting step, (ii) a jet spray wash with detergent (which may include egg brushing and egg rotation), (iii) a rinse and (iv) a drying step (Messens et al., 2011; Leleu et al., 2011). This is mostly an automated process reported to take less than a minute to complete. Small manual-based equipment is also available for low production egg-laying farms or backyard operations. Done correctly, washing reduces the egg surface's microbial load, minimising cross contamination and bacterial ingress into the egg.

In the European Union (EU), the European Commission Regulation (EC) No 589|2008 (Article 2) defines two classes of eggs. Class A eggs are sold as table eggs and have detailed specifications. All eggs not meeting these stipulated quality measures are Class B and are destined to be pasteurised and used in food processing. In the EU, Class A eggs should not be washed or cleaned in any way, pre- or post-grading. This is because cleaning can damage the cuticle and egg shell, potentially allowing bacterial ingress. For some EU member states, a modified requirement permits egg washing in certain packing centres following a code of practice. These eggs can only be sold within the member states where this requirement applies.

In the United States, the details of egg washing are: (i) wetting with a light spray of warm water to moisten and prepare the egg for debris removal, (ii) washing by spraying water at

32°C or higher (generally 11°C warmer than the egg temperature) with an alkaline detergent (pH 10–11) while using rotating brushes, (iii) rinsing by spraying with a sanitiser (generally 100–200 ppm chlorine) at the same temperature as the wash water, and (iv) air-drying. Storage and transport of eggs post-washing is required to be at 7.2°C (Hudson et al., 2016; U.S. Department of Agriculture, 2014). Ambient temperature washes are currently prohibited, even though washing at the higher temperatures (32–49°C) means eggs take longer to cool.

In Australia, egg washing is currently not a requirement. However, the sale of dirty eggs is prohibited under Standard 2.2.2 and Standard 4.2.5 of the Code. The majority of eggs produced in Australia are subject to some form of egg washing. Variability in egg washing practices across the Australian egg industry were highlighted in the survey results from a nationwide egg producers' workshop (Chousalkar et al., 2017). Most commercial egg washing machines in Australia have an egg contact wash time of around 30 seconds or less (Chousalkar et al., 2017).

Washing can remove the cuticle from the egg shell, making it easier for bacteria to enter the egg through pores in the shell. There is a greater risk of bacteria getting into eggs that are washed. Wang and Slavik (1998) reported the longer the storage time after washing, the higher the levels of egg penetration by *Salmonella*. Current commercial practice aims to minimise the time between laying, grading and packing, and delivery to the point of sale.

Water can facilitate *Salmonella* movement through the shell and beyond the reach of sanitisers. During washing, the way water is applied (e.g. temperature, duration, use of brushes/jets) and the water quality are critical.

Temperature can affect the egg pressure differential (i.e. the difference between pressure inside and outside the egg). A negative pressure differential can cause external matter to be 'sucked' into the egg. The temperature of wash solution must be kept above that of the egg and its contents. An egg will be at ambient or storage temperature immediately before it enters the washing machine. This should be considered to avoid thermal cracking or negative pressure differentials during washing. Similarly, final rinse water should always be slightly warmer than wash water. This prevents a temperature differential causing a negative pressure in the egg, which in turn would draw wash water into the egg contents (Hutchison et al., 2003).

Egg washing chemicals may alter the shell surface and damage the cuticle layer, increasing the risk of bacterial transfer into the egg contents (Australian Eggs Limited, 2025a; Gole et al., 2014b). Egg penetration by *S*. Typhimurium was reportedly higher in washed eggs than in unwashed eggs, possibly due to cuticle damage from washing (Gole et al., 2014b).

If eggs are not washed, dirty eggs can be cleaned using a dry abrasive method. A clean, dry, sanitised cloth or other suitable material can be used. Equipment used to clean unwashed eggs should be sanitised or disposed of daily (Australian Eggs Limited, 2010). Dry-cleaned eggs with visible soil or other matter that cannot be removed should be segregated and disposed of hygienically (NSW FA, 2015).

4.7.2 *Salmonella* vaccination

Vaccination plays an important role managing *Salmonella* infection of laying hens if used together with good sanitation, biosecurity and management practices (Aehle and Curtiss, 2017; Martelli et al., 2017). Vaccination of layer hens combined with good rodent control is effective in reducing egg-associated foodborne ST outbreaks in Western Australia (WA Health pers. comm.). Internationally, approaches vary and need to be considered in context of the full suite of management measures in place. The United States does not mandate

Salmonella vaccination for layer flocks. The European Union requires vaccination in member states with SE prevalence above 10%. The Code of Practice for British Lion eggs mandates vaccination of pullets against SE and ST (BEIC, 2025). Vaccines currently used internationally are intended to be administered to birds while they are at a hatchery (<16 weeks of age) (Neelawala et al., 2024).

There are two types of *Salmonella* vaccines widely used in layer hens: live attenuated and inactivated (Jia et al., 2020). Live attenuated vaccines contain laboratory-weakened versions of the original pathogen. They can produce strong antibody-mediated and cell-mediated immune responses and provide long-term immunity with only one or two vaccine doses. Inactivated vaccines produce a different response, generally only inducing antibody-mediated immunity. Multiple doses of inactivated vaccine are needed to build up and/or maintain immunity. Use of live or inactivated *Salmonella* vaccines only reduces the susceptibility to Salmonella and cannot create an impermeable barrier against infection (Gast, 2007).

In chickens, live *Salmonella* vaccines are generally more effective against both intestinal and systemic infection than inactivated vaccine preparations. This is largely because they stimulate both cellular and antibody-based immune system responses (Methner, 2018). To maximise protection, vaccination programs combining both live and inactivated vaccines are often used (Martelli et al., 2017).

Efficacy of vaccination may be limited by the vaccine delivery method (e.g. spray, oral or intramuscular injection), production system, biosecurity system, and presence of pests such as insects and rodents (Jia et al., 2020). In one study, hens vaccinated with different SE vaccination schemes during rearing had reduced immunity to SE at 82 weeks old (Van de Reep et al., 2018). This SE challenge study showed little difference in the infection outcome between vaccinated and non-vaccinated hens. In both groups, the majority of liver, caecum, spleen, and follicular fluid samples were positive for the challenge strain (Van de Reep et al., 2018). An improved immune response could be achieved by administering additional vaccine doses (Jia et al., 2020).

Multi-age farms are a particular challenge to the effectiveness of vaccination. Older *Salmonella*-infected birds in the shed may serve as a continuous source of bacteria for newly arrived pullets (Sharma et al., 2018). Continuous housing of birds in multi-age laying systems also hinders thorough environmental cleaning and sanitising (Jia et al., 2020).

Hens in vaccinated flocks can still become infected with SE and produce contaminated eggs. However, this happens at a lower frequency than would be expected in unvaccinated flocks kept in an SE-contaminated environment (Davies and Breslin, 2004). Vaccination alone without good hygienic practices and biosecurity measures cannot guarantee *Salmonella*-free flocks or eggs.

Although there are vaccines selective for SE, they are not currently approved for use in Australia. Scott et al. (2020) determined the efficacy of an SE PT7A vaccine in Australian flocks. It induced SE antibody levels for at least 47 weeks post vaccination and also reduced colonisation of caeca and the largest follicle (F1). However, the induced immune response was not able to reduce SE shedding at a statistically significant rate (Scott et al., 2020).

4.7.3 Feed additives

Literature demonstrates promising yet variable efficacy of feed-additive technologies in the reduction of *Salmonella enterica*, and more specifically serovar Enteritidis, in layer-type chickens. Feed additives can be classified into several main categories, such as probiotics,

prebiotics, organic acids, short- and medium-chain fatty acids, essential oils, and bacteriophages. Studies show these additives have the potential to modify intestinal microflora to enhance the overall gut health of the bird and reduce *Salmonella* colonisation.

Probiotics have been studied for their ability to outcompete harmful bacteria in a laying hen's gastrointestinal tract. *Bacillus* species additives have shown the ability to modify the composition of chicken gut microbiome while reducing *Salmonella* caecal colonisation (Khan and Chousalkar, 2020; Price et al., 2020; Oh et al., 2017). However, continuous or intermittent feeding of probiotics does not eliminate the pathogen (Khan and Chousalkar, 2020).

Postbiotics are non-viable microbial products or metabolic byproducts. Unlike live probiotics, postbiotics consist of short-chain fatty acids, bacteriocins, enzymes, and cell wall fragments. These additives are believed to have the ability to reduce Salmonella colonisation in poultry (Neelawala et al., 2024). Postbiotics such as the *Saccharomyces cerevisiae* fermentation product (SCFP) improve gut health by maintaining immune robustness and enhancing digestive efficiency. Chaney et al. (2023) evaluated the dietary inclusion of a SCFP additive on the SE colonisation of caecal and ovarian tissues of commercial pullets. Layer pullets were fed a control diet with or without a postbiotic feed additive and subsequently challenged directly or indirectly with SE at 16 weeks of age. The SE load in the caeca of birds indirectly exposed to the SE inoculation showed the postbiotic was associated with a significant reduction of SE-positive birds (50% vs. 72.5%; $p = 0.037$), 7 days after inoculation. However, no differences were observed between treatment cohorts for SE prevalence in ovary tissues of directly or indirectly challenged birds.

The yeast cell walls (YCW) of *Saccharomyces cerevisiae* are a prebiotic feed additive that can reduce *Salmonella* load through an agglutination mechanism (Hofacre et al., 2024). Mannan-oligosaccharides, present in YCW, and β-glucans have been identified as effective prebiotics that can inhibit the adhesion of *Salmonella* to intestinal epithelial cells, thereby reducing colonisation (Neelawala et al., 2024). In a study evaluating a commercial YCW preparation fed from day 1 to 17 weeks of age and week 10 to 17 weeks of age in layer pullets, authors reported no reductions in caecal or ovary tissue SE prevalence for any treatment (Hofacre et al., 2018). This study was later repeated and while a reduction in SE prevalence in the caeca of birds 7 days post challenge was observed, there was no difference in ovary SE infection rates (Hofacre et al., 2024). However, Girgis et al. (2020) demonstrated a statistically significant reduction in SE prevalence on ovary tissue of hens fed a different mannan-rich prebiotic product.

Organic acids, such as formic and acetic acids, and short- and medium-chain fatty acids have antimicrobial properties. These acids function by either inhibiting or reducing the distribution of *Salmonella* in the avian gut (Neelawala et al., 2024). Organic acids can cause intracellular acidification of bacterial cells, while fatty acids can enhance cell wall permeability and leakage. When combined with other ingredients, such as essential oils or probiotics, these feed additives have enhance antimicrobial activity (Abd El-Ghany, 2024). Upadhyaya et al. (2015) investigated the efficacy of caprylic acid, a medium-chain fatty acid in reducing SE colonisation and egg contamination in SE-inoculated layer hens. They found feed supplementation consistently decreased SE presence on the eggshell (from 60% to 14%) and in the yolk (from 43% to 10%). These results suggest that caprylic acid could potentially be used as a feed additive to reduce vertical transmission of SE.

Bacteriophages are a promising development in bacteria intervention technology because they specifically target species of interest for destruction. Internationally, there are a number of bacteriophage products that have shown the ability to greatly reduce, or even eliminate, *Salmonella* colonisation (Clavijo et al., 2019; Wójcik et al., 2020; Żbikowska et al., 2020). In

Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates feed medications, supplements and additives, and currently there are no bacteriophages approved for animal feed addition.

4.7.4 Shelf Life

As discussed in Section 2.14 the potential for growth of *Salmonella* spp. during an egg's supply chain is dependent on the YMT and the time and temperature experienced. Some countries mandate shelf life as part of managing SE growth in eggs (refer to SD3). In Australia, the Code does not prescribe shelf life for any foods; this is the responsibility of the producer or manufacturer of a food. Industry best practice under the Egg Standards of Australia (ESA) is 42 days or less from the time of packing and identifies eggs be used before the best before date. ESA notes the 42 day shelf life applies only to eggs stored and transported in the range of 4-18°C and in a manner to prevent condensation or contamination (Australian Eggs Limited, 2025b). For businesses under NSEMAP, eggs must be stored between 1-15°C. The 42-day recommendation stems from industry experience and relates to maintaining optimal egg quality, not to egg food safety issues (Australian Eggs Limited, 2025b). There are no legal requirements for eggs to have a best before date of 42 days, only that any shelf life set should be validated.

In 2015, a nationwide egg producers' workshop on ST in the Australian egg industry involving more than 80 commercial egg producers discussed the need to review guidance on table egg best before date (Chousalkar et al., 2017). At the time, SE was not a relevant issue for the Australian egg industry. The discussion focused on the possibility of ST survival on the eggshell surface of eggs for up to three weeks (Chousalkar et al., 2017; Gole 2014a).

Thomas et al. (2006) predicted storage at 16°C through the supply chain will allow growth of *Salmonella* in contaminated eggs after 18 days post farm-gate. This estimate is reduced to 10 days if eggs are stored at 22°C and 4.6 days if stored at 30°C. Thomas et al. (2006) concluded commercially produced and graded eggs in Australia may pose a potential risk to consumers if they are stored at 20°C for the maximum recommended shelf-life before consumption.

New Zealand modelled storage times and temperatures as part of a 2015 risk assessment (MPI, 2015). New Zealand's model was built with the assumption that a single cell contamination occurred post-lay and used growth rate prediction based on Thomas et al. (2006). Their outputs showed that 20% of contaminated eggs permitted logarithmic growth of *Salmonella* after 45.9, 28.1, 17.2, 10.5 and 6.5 days of storage at temperatures of 10, 15, 20, 25 and 30°C, respectively. Based on these results, New Zealand set shelf life requirements of 21 days at room temperature or 35 days if stored and held at 15˚C or less. New Zealand amended these shelf life requirements, after deeming them too harsh for the current *Salmonella* situation in the country, to 35 days from the date of lay regardless of temperature (NZFS, 2024).

The USA, which requires refrigeration throughout the egg supply chain, allows for voluntary use of a 'sell-by' date, which is used for the majority of eggs (some states may vary). If a sell-by date is used it may not exceed 30 days from the date of pack (USDA, 2025). In contrast to the USA, Europe does not require refrigeration of eggs through the supply chain. European legislation uses a 'date of minimum durability' to refer to shelf life. For eggs, Europe mandates this date not exceed 28 days from lay (EFSA BIOHAZ Panel et al., 2014). The British Code of Practice has similar standards for eggs, assigning a best before date of no more than 27 days from lay (BEIC, 2025).

4.7.5 Refrigeration

FSANZ (2012) excluded egg refrigeration during storage and transportation as a management measure when Standard 4.2.5 was developed. At that time, SE was not present in Australian laying flocks. The situation has changed over the past 12 years, and SE outbreaks, in people and in flocks, have occurred in multiple jurisdictions. With the emergence of SE-infected laying flocks in Australia, the current assessment considered through-chain refrigeration of eggs.

Salmonella contamination in and on eggs is influenced by the eggs' storage/transport temperature. Growth of most salmonellae is substantially reduced at <15°C and prevented at <7°C. Refrigeration through the egg supply chain has been a key general recommendation for mitigating the public health risk of SE in eggs (Gast et al., 2007). However, the effectiveness of refrigeration on preventing SE growth depends on several factors. These include the initial level and location of contamination, the potential for bacteria or nutrients to move within eggs during storage, and the rate at which eggs are cooled.

Condensation ('sweating') caused by moving eggs from 4°C to ambient (i.e. lower to warmer) temperatures provides opportunity for potentially contaminated surface moisture to move into the egg shell. As moisture is needed to allow penetration, any stage of production where both moisture and a positive temperature differential may be present provides an opportunity for bacterial invasion. Consequently, once eggs have been held at a certain low temperature (i.e. such as at or below 15°C), they should be handled at that temperature through chain to avoid condensation, noting the closer to refrigeration, bacterial growth is prevented. Industry quality assurance practices aim to prevent temperature changes causing condensation to form on the egg surface. This risk of enabling bacterial invasion through condensation forming can be mitigated by proper egg washing (see section 4.7.1).

Storage conditions affect survival and growth of SE in eggs. Improper storage conditions, including temperature abuse or long-term storage, can increase the risk of SE growth. SE can survive without growth at refrigeration temperatures, but can grow rapidly above 20°C. Humphrey et al. (1993) found SE could grow in the yolk and albumen of eggs stored at 25°C, but not at 10°C. Cox et al. (2000) found SE could grow in the yolk and albumen of eggs stored at 20°C, but not at 15°C. Refrigeration at less than 5°C prevents SE growth in eggs. Schoeni et al. (1995) found SE growth was significantly reduced in eggs stored at 4°C compared to eggs stored at 10°C. Experimental data demonstrated prompt refrigeration of eggs is an essential risk reduction practice for preventing egg-associated disease transmission to humans (Gast et al., 2010).

High humidity can increase the risk of eggshell contamination and promote SE growth. Messens et al. (2006) found SE could survive for longer periods on eggshells stored in a high-humidity environment compared to a low-humidity environment.

SE growth is limited in the albumen (DeWinter et al., 2011) but once it reaches the yolk, very rapid growth can occur under permissive temperatures. Bradshaw et al. (1990) evaluated the kinetics of growth of SE in yolks of eggs incubated at 37, 15.5, or 7°C. At 37°C, with an initial inoculum of about 1 CFU/g of yolk, SE multiplied with a generation time of about 25 min and reached a concentration of about 10^8 CFU/g in 12 h. When similarly infected eggs were incubated at 15.5°C, SE multiplied with a generation time of 3.5 h, and reached a density of about 10^2 CFU/g in 24 h, and 10^4 CFU/g in 48 h. Cell density of $>10^7$ CFU/g was reached in 4 days at this temperature. Eggs incubated at 7°C had no SE growth observed up to 94 days.

As long as the vitelline membrane is strong, SE cannot access nutrients present in the yolk and bacterial growth remains low. Egg age, temperature, humidity, and handling of the eggs affect the quality of the membrane and should be managed.

In Australia, industry guidelines recommend eggs are stored below 15°C as soon as possible after collection and washed within four days of being laid (Australian Eggs Limited, 2025a). There are no requirements or prescriptive guidelines on egg storage in supermarkets. It is not uncommon to find eggs kept at ambient temperature in stores. Current industry practice is to label cartons, recommending eggs are stored under refrigeration once purchased.

Overseas, refrigeration requirements vary. In the United States, eggs must be held and transported at or below 7.2°C (45°F) beginning 36 hours after laying (FDA, 2009). In the EU, according to Regulation (EC) No. 853/2004, “eggs should be stored and transported preferably at a constant temperature, and should in general not be refrigerated before sale to the final consumer (EU, 2008). This is because “cold eggs left out, at room temperature, may become covered in condensation, facilitating the growth of bacteria on the shell and probably their ingression into the egg.”

4.7.6 Traceability

Many countries have legislative requirements for through-chain traceability. Codex principles for traceability set out a one-step forward (where the food went), one-step back (where the food came from) approach (CAC/GL 60-2006; Codex Alimentarius Commission, 2006).

In responding to a food safety incident, however, a one-step approach can be slow and cumbersome, leading to delays in traceback investigations and an increase in illnesses. To enable a faster response time, greater visibility through the supply chain can occur by tracing back and forward further than one step. An effective traceability system enables government agencies, food producers, distributors and retailers to act swiftly. This keeps potentially unsafe food from endangering consumers, while reducing industry costs of food recalls (NSWFA, 2022).

In Australia, Standard 4.2.5 requires a similar approach to Codex recommendations for traceability. An egg producer must not sell eggs unless each individual egg is marked with the producer’s unique identification. The producer does not have to mark their eggs if they are sold or supplied to an egg processor who subsequently marks the eggs with the producer’s or processor’s unique identification before sale. The egg producer must have a system to identify to whom the eggs were supplied. Additionally, the egg processor must have a system in place to identify from whom the eggs were received and to whom they supply. An identifying mark is mandatory, but a date and/or batch number are recommended to further enhance traceability.

The information on the egg stamp should be considered. A traceability review in 2015 found date and/or batch information combined with a unique identifier would be needed if locally acquired SE emerged in Australia (NSWFA, 2022). This information would help limit the extent of SE outbreaks and facilitate rapid trace back and quarantine measures. Enhancing egg traceability was highlighted during the 2018–2019 SE outbreak in NSW where 17 infected premises were implicated (NSWFA, 2022). Information that helps identify the farm of origin is usually available on the egg carton. However, public health officials noted in their investigations following illness, consumers often discarded the egg carton prior to storage at home. Once removed from their packaging, the lack of traceability compounds difficulties in investigating egg-related foodborne illness outbreaks (Szabo et al., 2020).

Eggs can be stamped at the farm where they are produced or at a grading facility, noting only one move from the farm is allowed for an unstamped egg. Issues may arise if there is movement of unstamped, ungraded eggs through the supply chain. For example, traceability is hampered if unstamped eggs are traded between wholesalers before going to processing or food service. The 2018–2019 SE outbreak investigation found eggs had been moved unstamped after they left the business to which the original farm had sold them. Three properties had moved unstamped, ungraded eggs to other grading facilities, some of which also housed laying hens (NSWFA 2022).This meant unstamped eggs moved further than one step from the original layer farm, adding to the difficulty of tracing eggs to the farm.

4.8 Salmonellosis risk factors associated with production, processing, distribution, preparation and consumer handling

Stage of production	Description	Risk factors and their impact
Parent breeder	Breeding farms house hens and roosters to produce fertilised eggs. The fertile eggs are collected daily and transported to the hatchery. Breeder stock are retained for around 12 months and then sent to meat processing.	Contaminated birds, environment, and eggs may result in *Salmonella* transferring to hatcheries.
Hatchery	Fertilised eggs are incubated in hatcheries. Day-old chicks are screened and sexed before being vaccinated against avian diseases such as infectious bronchitis virus (IBV) and Marek's disease (MD). Day-old chicks are then transported to farms for rearing.	Contaminated environment, and eggs may result in *Salmonella* transferring to hatcheries.
Pullet rearing	Day-old chicks are reared in either deep litter or cage-rearing systems until about 17 weeks old. They (pullets) are then transferred to layer farms (either the same farm or sold on to others). Pullets are vaccinated against endemic poultry diseases such as fowl cholera, avian encephalomyelitis (AE) and Newcastle disease (NDV).	*Salmonella* infected day-old chicks or replacement pullets can be a source of layer flock contamination.
Layer farm	Layers remain in production systems generally from about 18 until 78 weeks old. In most systems, birds are considered spent between 72 and 80 weeks old. Layer farms vary greatly in size, from small farms with a few thousand birds to larger operations with >500,000 birds.	Vertical transmission to eggs via the reproductive tissue of infected hens. Horizontal transmission from infected hens or the environment. Contaminated feed and water. Transmission of *Salmonella* from contaminated or infected pests. Bird health associated with stress and moulting. Contamination from external farm visitors. Shed conditions such as

Stage of production	Description	Risk factors and their impact
		presence of dust, yolk, water, and faeces.
Egg collection	Eggs are generally collected daily, transported either manually or by conveyer belt, to an on-farm packing shed or a centralised grading facility. Cracked or visibly dirty eggs are generally disposed of or collected for further processing.	Time between when the egg is laid and its collection, enabling growth of *Salmonella*. Surface contamination of egg collection systems or containers. Environmental conditions, health and hygiene of personnel.
Egg cleaning	Eggs are then either wet or dry cleaned. On commercial farms, eggs are wet washed—a mechanised process of spray wetting, followed by ultraviolet sanitising, rinsing and drying. Dry cleaning is mainly only done on smaller farms, as it is labour intensive.	Opinion is divided on the benefits of washing eggs. Wet washing may remove or damage the protective cuticle, exposing pores and increasing the risk of bacterial penetration. Dry washing can spread surface contamination of eggs. Temperature, wash cycle, chemicals, drying parameters and contaminated washing equipment. Cross-contamination of eggs from dirty water and damage during washing.
Egg grading	Eggs are placed into plastic or cardboard fillers and checked for defects, cleanliness and quality. Modern egg grading equipment uses bright lights to inspect the internal quality of an egg—a process known as candling. A visual assessment may follow. Automatic acoustic crack detection technology may be used to identify cracks, micro-cracks or fractures. Dirty or cracked eggs are diverted for either disposal or further processing. Eggs are also graded by size for market specifications.	Candling or auto crack detection cannot identify internal or external microbiological contamination. Grading equipment may facilitate cross contamination. Equipment contact with washed and unwashed eggs. Environmental conditions.
Packaging	Eggs are weighed and sorted into different sized cartons. Packaging helps prevent damage and breakage. Eggs are packaged for retail in clean	Reuse of contaminated egg cartons or plastic fillers may result in cross-contamination.

Stage of production	Description	Risk factors and their impact
	moulded fibre or plastic cartons to prevent damage.	Inappropriate packaging may increase breakage.
Storage and transport	Eggs are stored between laying and grading / washing, after grading and during transport to retail.	Storage conditions (time, temperature and humidity) can affect the growth of microorganisms that may be present on or in the egg. Inappropriate transport and storage equipment. Presence of yolk and/or moisture on outside of egg.
Further processing	Excess or second grade eggs (e.g. cracked or soiled) are often diverted to further processing steps for the manufacture of egg products such as liquid and dried egg.	Contamination of egg pulp. Ineffective pasteurisation.
Sale of eggs	Primary producers, wholesalers, retailers may be involved in the sale of eggs.	Sale of cracked or dirty eggs. Time and temperature of storage between lay, sale, and consumption if eggs are internally contaminated by *Salmonella*. Inadequate recall processes.
Food service and consumer storage and handling	Consumers from a wide demographic range purchase eggs. Storage at 5°C or less in consumers' homes or food service businesses has been recommended by industry.	Time and temperature of storage. Ineffective stock rotation, hot-holding of egg foods, cooling and re-heating of egg products. Preparation of raw or low-cooked egg products, especially for vulnerable populations. Cross contamination of the preparation or storage environment.

4.9 Exposure assessment quantitative modelling

Questions about through-chain egg temperature or storage time are best answered using quantitative models of egg supply chains. A quantitative model simulates the various stages of egg production, distribution and consumption, estimating the likelihood of contamination and subsequent illness under different scenarios. Further, the model allows investigation of interventions such as environmental monitoring, passive human surveillance, and temperature control in different parts of the supply chain.

The P301 risk assessment used the results for a quantitative model developed for Australian Eggs Corporation Limited (AECL) (Thomas et al., 2006). This model was subsequently modified by the European Food Safety Agency (EFSA) to evaluate spoilage of eggs in Europe (EFSA, 2014). The ANSES/BfR model has now been adapted for Australian egg supply chains under this proposal (refer to SD2 for details).

The conceptual model used for the risk assessment is presented in Figure 6 and Figure 7 and considers seven major sections:

- layer farm
- egg supply chain
- preparation, consumption and illness
- laboratory testing and reporting
- epidemiological investigation
- traceback and farm identification; and
- recall

Section 4.2 (Contamination of eggs during formation) highlights the importance of SE colonisation of the reproductive tissues of hens resulting in internal contamination of eggs during formation. The first part of the Exposure Assessment model considers when and how many SE contaminated eggs are laid each day. The presence of SE within an egg depends on the hen being infected with SE. In this model, only an SE-positive hen can lay a SE-positive egg; not all eggs are SE-positive.

To effectively answer the questions posed in this risk assessment, it is important to note SE is not endemic in Australia. Utilising quantitative models from other regions of the world where SE is endemic without adjustment would result in unrealistic conclusions. The models developed in this risk assessment are therefore tailored to a single small or medium-sized farm becoming SE-positive during a production cycle. The initial steps in determining exposure include identifying the time at which a farm becomes SE-positive during the egg-laying cycle, duration for hens to become infected, and onset of SE-positive egg production.

If an egg is internally contaminated with SE during formation, potential growth depends primarily on time and temperature during storage. Higher temperatures and longer storage times will degrade the protective mechanisms within the egg to inhibit SE growth. The model considers temperatures and times which an egg may experience through the supply chain.

An outcome of the investigations into the 2018/19 SE incident was an appreciation of the complexity and diversity of egg farms and egg supply chains. The sharing of ungraded eggs between producers and use of feeder farms increased the complexity of traceback investigations.

Modelling the vast array of combinations of egg farms and supply chains is an unrealistic endeavour. FSANZ made a number of simplifying assumptions on what will be modelled:

- a single farm
- only small and medium size farms
- a simplified supply chain

Evidence from veterinary epidemiological investigations of the 2018/19 SE incident indicates SE can spread between properties through sharing of eggs, equipment, vehicles and people. Hatcheries and dispersal of infected chicks and pullets were not identified as sources of SE. The exact way a primary farm in an infection chain becomes SE-positive is often unknown.

Eggs are sold and prepared in homes or food service settings. They can be served in various ways, either as individual egg meals or as ingredients in dishes consumed by many people. Most foods are cooked during preparation, which can reduce or eliminate any SE cells introduced through the egg. However, cooking temperature and time can vary greatly, and some foods, including desserts, may not be cooked at all. The model at the end of the preparation stage, estimates the number of SE cells each person might ingest when consuming a food containing contaminated egg.

A dose-response model (see Section 3.2) is used to predict if a person exposed to the food becomes ill. If illness occurs, the model continues by considering health care-seeking behaviour, such as whether the person seeks medical support from a doctor, if a stool sample is requested and tested, and if a positive result is reported to a health department.

If multiple notifications of locally-acquired SE infection of the same strain are reported, an epidemiological investigation will be initiated. The model applies two rules for the start of investigation: (1) an outbreak of two or more notified cases linked to consumption of the same egg (i.e. same food source), or (2) three or more notified cases that are unrelated linked by whole genome sequencing. The model assumes that all epidemiological investigations successfully identify a suspected positive farm.

Next, responsibility for investigation shifts from the health department to primary industries and/or food regulatory departments for confirmatory testing and to halt movement of eggs from farm. Finally, a recall is initiated with FSANZ, and consumption of eggs stops. This concludes what is referred to as the ‘base model’.

With the completion of the base model, necessary adjustments are made to consider specific scenarios related to environmental testing and through-chain temperature control.

Environmental testing

To illustrate applying the model, two environmental testing regimes were considered: (1) a single test per production cycle at peak production, and (2) regular tests every 13 weeks. As with supply chain scenarios, there are many possible combinations of testing regimes, such as including a test at the start and/or end of production cycle. For simplicity, it is assumed environmental testing is always successful at detecting a positive farm.

Without a start or end of cycle environmental test, a farm may introduce negative pullets into a positive environment or sell positive spent hens to other farms. The model assumes a farm becomes positive at a random time during the production cycle. If a farm becomes positive after a single test (testing regime 1) or after the last test of a cycle (testing regime 2), the farm will remain positive until the first test of the next production cycle or when the passive human surveillance system identifies enough notified cases of illness and initiates an epidemiological investigation.

A farm being positive at the start of the next production cycle has implications for assumptions about when SE-positive eggs begin to be laid. Evidence from infected farms in Australia suggested that there may be a period of time between the introduction of SE into the layer environment and a serological conversion. A fixed time of 42 days was chosen. Although, too late for inclusion into the quantitative model development, the paper by Thomas et al. (2009) describes experimental studies and a model developed for the horizontal spread of SE between hens in a flock. Although the individual model predictions for the number of infected hens is highly variable, the predicted time for half of a flock to be infected is similar to the value used in the quantitative supply chain model.

For the second production cycle, it is assumed the between-flock clean-up is not sufficient to eliminate SE from the layer environment, resulting in many immature pullets becoming infected when introduced on farm. Consequently, SE-positive eggs are laid from onset of laying.

Temperature control

The second group of scenarios relates to through-chain temperature control. Evidence gathered during this proposal supports that refrigeration (approximately 5°C) is not widely used on Australian layer farms. This is likely related to the widespread practice of washing eggs. Cold eggs would need to be warmed to avoid thermal cracking when washed in warm water. Consequently, temperature scenarios started from the transportation step after grading, whether for direct sale, retail sale, or food service. It is acknowledged many farms hold eggs until grading or eggs post-grading between 12°C and 15°C. Eggs Standards Australia requires eggs to be held under temperature control below 15°C.

Storage temperature scenarios begin from transportation after grading. Information obtained from the major retailers in Australia confirm that eggs are transported and stored in distribution centres under refrigerated conditions. Front of store display is mixed between ambient room temperature and chill storage. Information for the wholesale food service supply chain was lacking. It was decided to consider this storage temperature after grading has scenarios with either refrigeration or ambient room temperatures. There was insufficient evidence to assign probabilities to either option.

Within the model (see SD2 for more information), pre-grading stages of on-farm before collection (stage 1) and on-farm between collection and start on-farm storage (stage 2) have a temperature distribution with a minimum value of 20°C, a mode of 24°C and a maximum of 28°C. This distribution is used for both supply chain temperature scenarios in the model (i.e. refrigeration and ambient). For a supply chain with ambient conditions the stages from on-farm storage after collection (stage 3) to storage before grading (stage 5) along with post-grading storage (stage 7) transportation to supply-chain pathway (stage 8) have a temperature distribution with a minimum value of 12°C, a mode of 15°C and a maximum of 18°C. Grading at ambient temperature (stage 6) and the stages for direct sale storage (stage 9), retail storage (stage 10), transportation to home (stage 11), transportation to food service (stage 12), food service storage (stage 13) and home storage (stage 14) have a temperature distribution with a minimum value of 18°C, a mode of 22°C and a maximum of 25°C when an ambient supply chain scenario is active. Note that within the model home storage has a 93% probability of occurring at refrigerated temperatures. This follows evidence from the social science assessment of consumer handling and consumption (see SD6), which found refrigeration in the home is very common.

The exposure model was developed using the R language (R Core Team, 2024) expanding on code prepared by Desvignes et al. (2019).

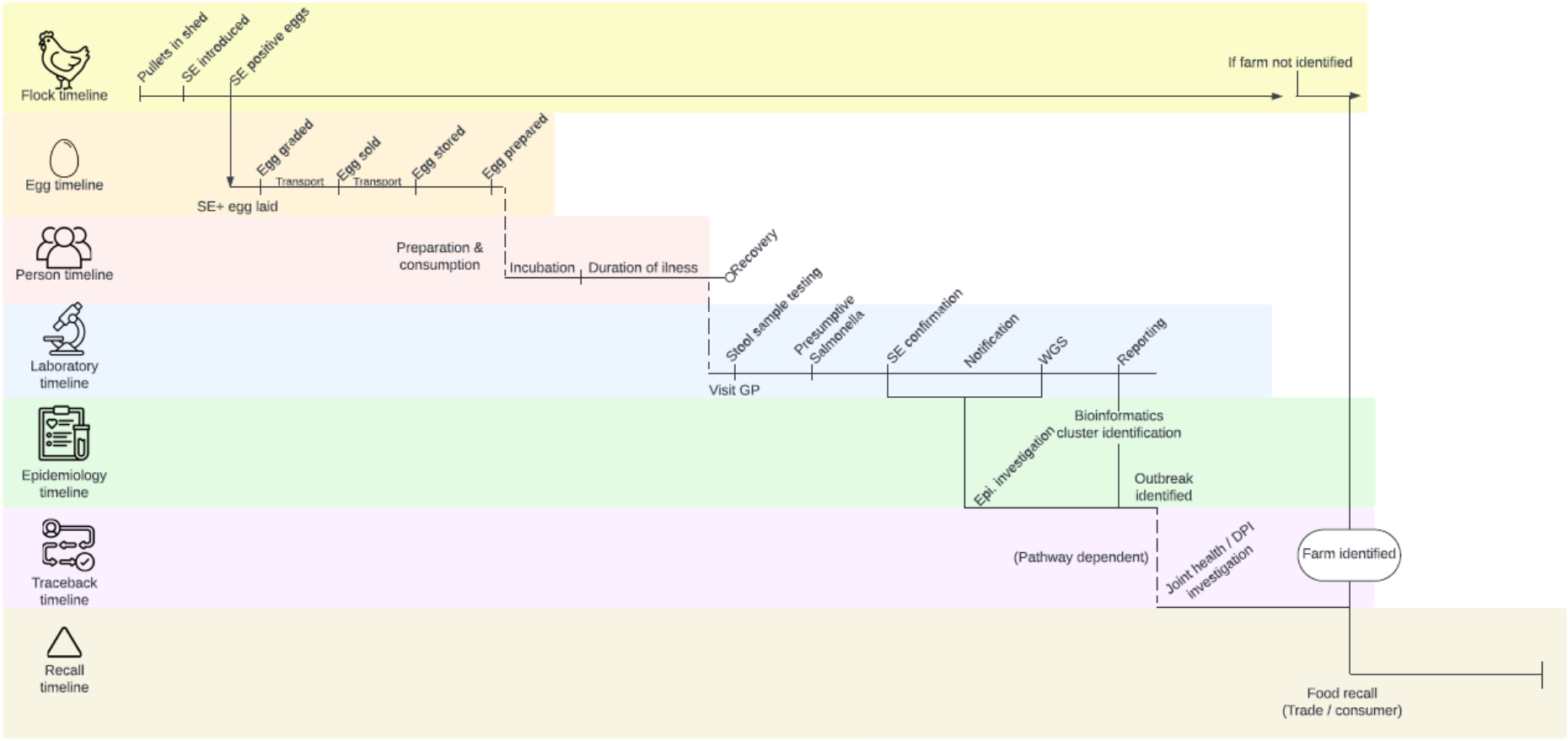

Figure 6: Conceptual model for the egg supply chain

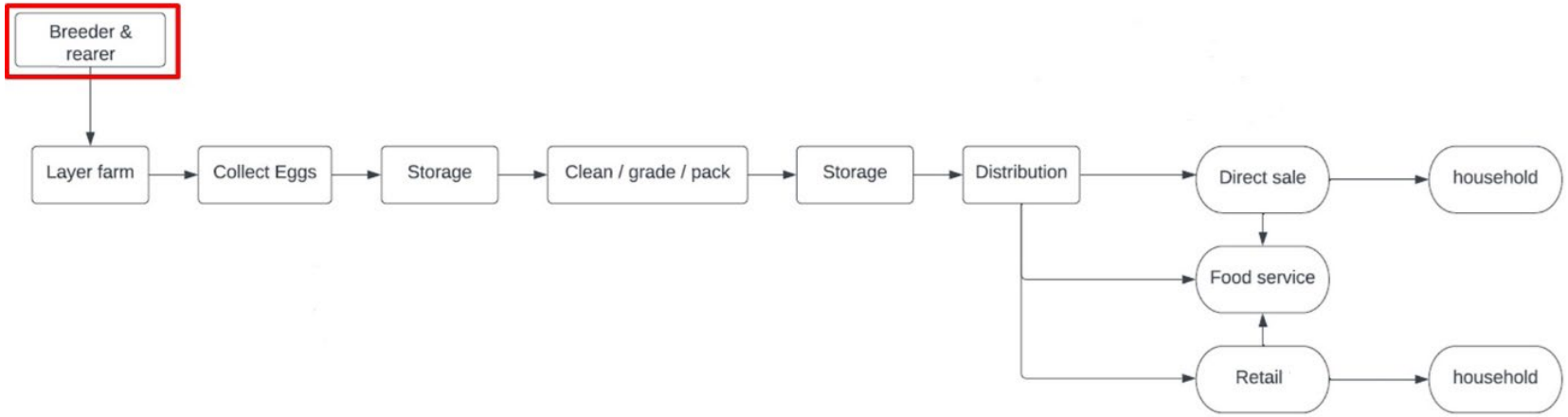

Figure 7: Schematic for egg supply chain from lay through the post-grading storage to preparation in food service or home setting

5 RISK CHARACTERISATION

Summary of risk characterisation

FSANZ developed a through-chain quantitative model to simulate stages of egg production, distribution and consumption, estimating the probability of contamination and subsequent illness under different scenarios (size of farm, on-farm monitoring, supply-chain time and temperature). The model outputs several metrics, including number of SE-positive eggs, actual illnesses, notified illnesses, outbreak events, and effectiveness of interventions (i.e. illnesses avoided).

Incorporating variables reflecting the current Australian SE status (i.e. sporadic occurrence) the model was used to determine the impact of two of the proposed mitigation measures on SE-related human illnesses: (1) on-farm environmental monitoring (i.e. testing) and (2) egg storage temperature after grading. Environmental monitoring regimes included one test per production cycle at peak production or regular testing every 13 weeks. Supply chain temperature scenarios include ambient or refrigeration temperatures.

Environmental monitoring

Until recently, passive human surveillance (PHS) (i.e. epidemiological investigation of notified cases of human illness) has been the primary means of detecting SE on farms. Apart from not protecting public health and safety, using human illnesses as sentinels is fraught; it relies on people seeking medical support coupled with effective traceback to a source farm. Moffat et al. (2016) reported, where a *Salmonella* investigation was initiated, only 68% result in identifying the specific farm producing the implicated eggs.

Implementing on-farm environmental monitoring reduces the number of illnesses associated with SE-positive egg layer farms, in both a small (1,000 hens) and medium (20,000 hens) sized farm. It also increases the chances of early detection of a positive farm. Testing every 13 weeks per production cycle is more effective at preventing SE related illness compared to a single test. Regular testing increases the likelihood of detecting SE on-farm before any illnesses occur. Using a single test may also mean a positive farm is undetected and continues supplying positive eggs into the second cycle.

Refrigeration

Refrigeration of eggs from both small and medium size farms, through the supply chain greatly decreases SE-related human illness. Refrigeration prevents growth of many microorganisms including SE if present in egg contents. A producer, processor and/or retailer can have more confidence in preventing foodborne illnesses when through-chain refrigeration is in place.

When implemented in tandem, environmental monitoring and refrigeration result in the greatest reduction in illness.

Finding the SE positive farm

The FSANZ model shows even with perfect traceback, an SE-positive small farm with a refrigerated supply chain is likely to go undetected by the public health system in a single production cycle; while human illness cases may be low, the farm remains a source of SE

that can potentially spread. Further, as the number of notified illnesses are typically few and sporadic, they may not trigger epidemiological traceback investigations. This means without environmental monitoring, small farms may only be detected when there is a major foodborne illness outbreak. For an SE-positive medium sized farm, the model predicts a 100% likelihood of detection by PHS by the end of the second production cycle, irrespective of the supply chain scenario (i.e. ambient or refrigerated).

The final component of the risk assessment is risk characterisation, which integrates hazard identification, hazard characterization, and exposure assessment into an estimate of the adverse effects likely to occur in a given population. In this risk assessment, the general population is considered.

As outlined in the exposure assessment (see section 4.8), a quantitative model (further described in SD2) was developed to understand the risks associated with a single, SE-positive, small (1,000 hens) or medium-sized (20,000 hens) farm that is producing SE-contaminated eggs. That is, the farm is already positive and model is specifically investigating an SE-positive egg and its potential to cause illness; it is not investigating the probability of a farm to become positive.

The model incorporates temperature profiles across 14 stages of the egg supply chain, from on-farm activities to food preparation, consumption, illness and public health response. The stages and their associated risk factors interact to define multiple supply chain pathways representative of what exists in Australia. Illness is simulated at the individual level for each serving, allowing for the estimation of number of foodborne illness cases from a single egg. Public health response is based on notified cases of foodborne illness and reporting of genomic data. The time taken for successful detection or identification of a positive farm is incorporated. Each iteration of the model stops with the initiation of an egg recall e.g. ceasing egg supply from farm.

The model simulates a single farm (be it small or medium in size) over 1000 iterations where each iteration represents a combination of slightly different conditions on that farm including: the date of SE incursion into the layer environment; and the temperatures and time each SE-positive egg is held at up to preparation and consumption. Each iteration is also subjected to mitigation scenarios: (1) on-farm environmental testing - either once per production cycle, or every 13 weeks; and (2) use of refrigeration (2-8°C) or ambient temperatures during egg storage and transportation after grading.

FSANZ understands all large farms in Australia are accredited under the voluntary SE monitoring scheme and follow industry schemes for egg production. As such, these farms have already implemented measures being proposed under P1060; there is little value in modelling the impact of introducing the proposed measures when they are already in place.

5.1 The base model: identifying the farm

Details on the model design and development are in SD2. The model is used to test the impact of environmental monitoring and temperature separately and then combined, as shown in Table 2. Modelling these 12 scenarios enables investigation of the effectiveness of proposed measures individually and together.

Farm size	6 scenarios at ambient temp	**6 scenarios at refrigeration**
Small	PHS & A	PHS & R
	PHS + 1 test & A	PHS + 1 test & R
	PHS + 13 week & A	PHS + 13 week & R

Medium	PHS & A	PHS & R
	PHS + 1 test & A	PHS + 1 test & R
	PHS + 13 week & A	PHS + 13 week & R

Table 2: Summary of 18 scenarios modelled: PHS = public health surveillance; +1 test = one environmental test applied; +13 week = quarterly testing at approximately every 13 weeks; A = ambient temperature supply chain; R = through-chain refrigeration

The base model considers when an SE-positive farm is identified by passive human surveillance (PHS) to initiate epidemiological investigations based only on notified cases of human illness. Notified illness cases means a person becomes ill following the consumption of food prepared from an SE-positive egg; the person then seeks medical attention, a stool sample is tested; and the SE-positive result is reported to a health department (Figure 6). For an outbreak (i.e. two or more notified cases linked to the same egg), an epidemiological investigation commences immediately and under the model, the farm is identified. Alternatively, a single, sporadic case of illness on its own would not trigger an investigation or lead to the successful identification of a farm. For individual cases of illness, a rule was developed where successful identification of a farm is made only after three cases are linked by genomic analysis. Linking cases of illness starts after public health units interview sick individuals and identify the cause as SE. Clinical isolates undergo whole genome sequencing (WGS) with potential clusters identified via bioinformatics. Identified case clusters are then reported to state and territory health departments for further investigation. The model simulates the progress of a traceback investigation over approximately 30 days; farm identification is not instant and follows the best case scenario for a public health investigation. The model assumes perfect traceback, meaning once the investigation starts it will not fail in identifying the contaminated farm. It should be noted, in reality investigations are often unsuccessful. Moffat et al. (2016) reported only 68% of *Salmonella* traceback investigations result in identifying a specific farm from which the implicated eggs had been produced.

If the farm is not identified as SE-positive by PHS in the first production cycle, then the model continues into the second production cycle. During the second production cycle, the farm is assumed to be SE-positive at the beginning of egg production. The second production cycle proceeds within the model the same as with the first cycle. If the farm is not identified as SE-positive by PHS in the second production cycle, then the model continues into the third production cycle. Similar to the second cycle, during the third cycle the farm is assumed to be SE-positive from the beginning of egg production. The model is currently built to end after three production cycles, even if the farm has not been identified by PHS.

Specific scenarios relating to environmental testing and through-chain temperature control were then considered.

5.2 Detecting the farm using PHS: impact of refrigeration

FSANZ sought to understand in small and medium size farms, the impact of through-chain temperatures on the ability to identify a positive farm when only PHS is occurring.

To investigate this, the model determined the likelihood of an SE-positive farm being identified after each production cycle under different scenarios. Figure 8 presents the results for four farm size and storage temperature combinations: medium-sized farm + ambient temperature (blue); medium-sized farm + refrigeration (red); small farm + ambient temperature (green); small farm + refrigeration (black). The value ‘0’, for ‘Production cycle’, represents farms at the start of their first production cycle.

For a small farm that implements ambient storage and transportation temperatures during

distribution, there is a 49% likelihood the farm will be identified through PHS after the first production cycle. This increases to 96% after two production cycles and 100% after three production cycles. A feature of the small farm model is the assumption that eggs are only sold and consumed locally. The shorter supply chain compared to the medium farm limits the possibility of internal growth of SE in the egg contents.

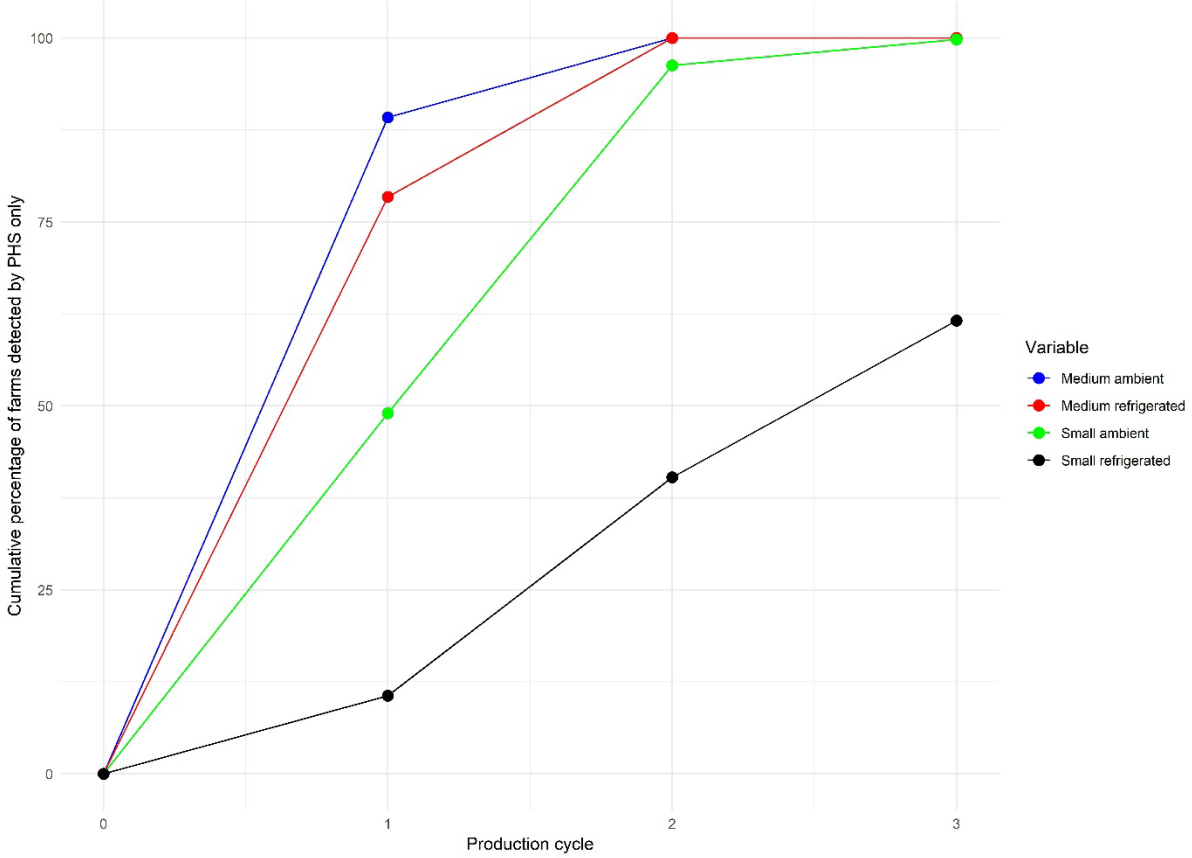

Figure 8: Cumulative percentage of egg farm (both small and medium) identification by PHS: by temperature storage conditions, over multiple production cycles

At the end of the first production cycle, the model estimates there is only an 11% chance for an SE-positive small farm with a refrigerated supply chain to be detected by PHS alone (Figure 8). This rises to 40% after two production cycles, and 62% after three completed production cycles. Given a production cycle is 64 weeks in length, by the end of the third cycle, the farm has potentially been SE-positive for up to 200 weeks (almost 4 years). Relying on PHS alone is an ineffective and inefficient means of identifying SE-positive farms.

The reason for the low chance of detection for a small farm with refrigeration through-chain reflects the combination of the small farm's shorter supply chain and through-chain refrigeration, which together limit the potential for internal growth of SE in contaminated eggs. Under this scenario, fewer contaminated eggs means fewer cases of foodborne illness. With fewer cases, it is also less likely the public health system would see notified cases of illness to trigger epidemiological investigations during the first production cycle. A farm relying on its short supply chain, refrigeration and PHS, delivers fewer cases of foodborne illness (compared to a small farm with an ambient temperature supply chain) but does not eliminate the farm from being an SE reservoir.

In subsequent production cycles of an SE-positive farm, incoming flocks are likely to be

infected earlier in their life cycle. This leads to earlier SE-positive egg production, more contaminated eggs entering the supply chain and more foodborne illness. The increased likelihood of the positive farm being detected in subsequent cycles is an indicator of increased foodborne illness and thus a higher probability of public health investigations. The risk of foodborne illness increases with subsequent flock cycles if the farm environment is not effectively cleaned between flocks. Further, presence of SE in the environment is a reservoir and potential source of SE contamination beyond that farm (as seen in the 2018-2019 SE incident in NSW).

An SE-positive medium size farm produces more contaminated eggs than a small farm leading to more illnesses; this directly relates to a larger farm producing more eggs. With more illnesses, PHS is more likely to trigger investigations; the model estimates an 89% chance of identifying medium-sized farms with an ambient temperature supply chain, and 78% chance for medium-sized farms with a refrigerated supply chain after one production cycle. There is a 100% chance for a medium-sized farm to be identified by PHS alone before the end of the second production cycle, irrespective of supply chain temperature. However, it is not appropriate to rely on cases of foodborne illness as the trigger for detecting SE on a farm; if an epidemiological investigation leads to farm identification and egg recalls, the system has not protected human health.

5.3 FSANZ Model scenario testing: impact of environmental testing

Three scenarios for predicting cases of illness from the consumption of SE-positive eggs for small and medium-sized farms with active environmental testing were considered:

1. Passive human surveillance only
2. Passive human surveillance with one environmental test per cycle
3. Passive human surveillance with regular 13-week environmental tests during the production cycle

5.3.1 Impact of environmental testing for a small farm with refrigerated supply-chain

Figure 8 showed the cumulative percentage of SE-positive farms (for both small and medium-size farms) identified through PHS and by supply chain temperature (ambient or refrigerated). For the medium farm scenarios, the farms were successfully identified before the end of the second production cycle, irrespective of the supply chain temperature. All iterations of the small farm with an ambient supply chain storage temperature were identified before the end of the third production cycle, while over a third of the time, the small farm with a refrigerated supply chain was not identified by PHS.

The introduction of environmental testing into the model for the small farm with refrigerated supply chain are presented in Figure 9. The PHS only scenario (black dots) is the same in Figure 8 and Figure 9. The introduction of a single environmental test achieved a modest improvement with 16% of farms identified, compared to 11% for PHS only. The regular 13 week testing achieved around 80% of farms being identified in the first production cycle. All farms were identified by the end of the second production cycle when environmental testing was used, compared to 40% for PHS only.

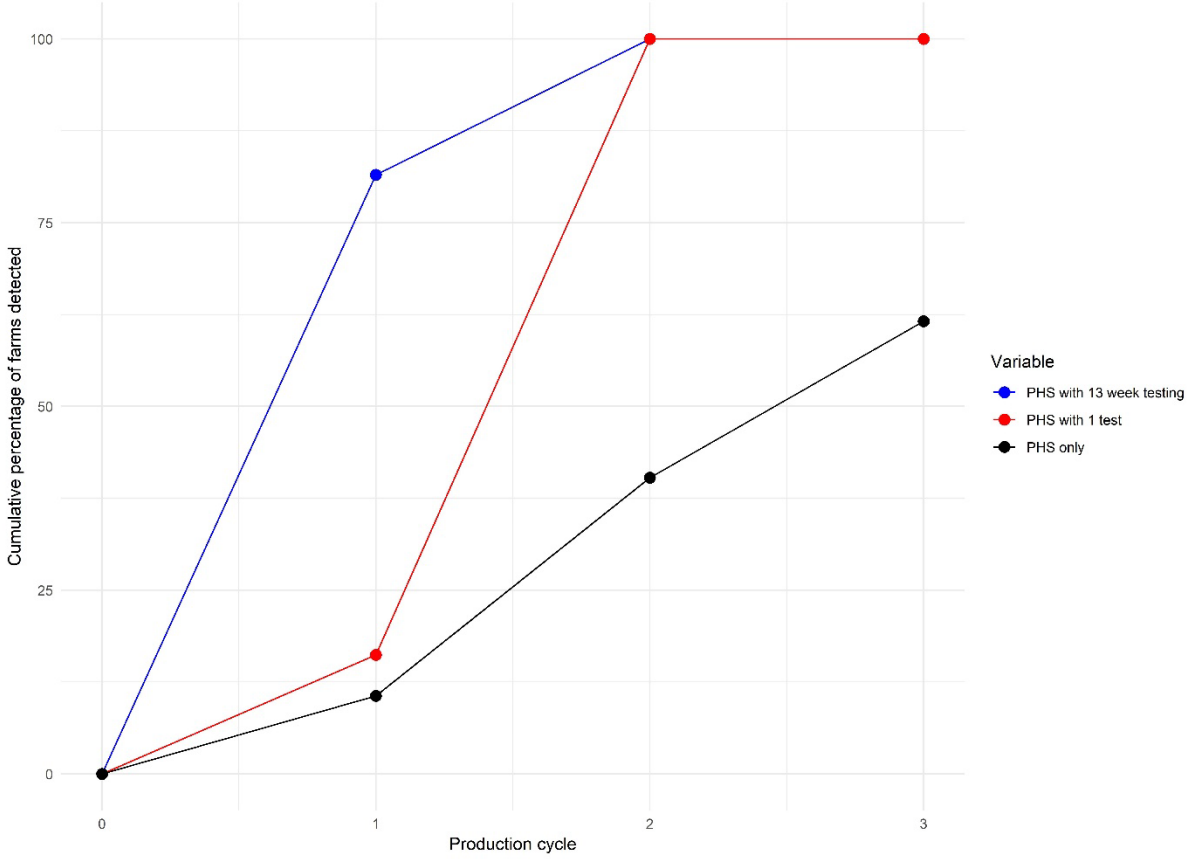

Figure 9: Cumulative percentage of identifying a small farm with refrigerated supply-chain over multiple production cycles: by PHS & 13 week environmental testing; PHS with 1 environmental test per production cycle; PHS only.

5.4 Modelling example for a small farm with refrigeration iteration #1

The quantitative model described in Section 4.9 (and refer to SD2 for more details) was developed for a small or medium-sized farm. For each of the environmental testing and supply chain temperature scenarios, the model was run 1,000 times. The large number of iterations is required to understand the interactions between temperature and time through the supply chain and how they affect public health, while also providing confidence in the results. The results presented below are the outputs of these 1,000 runs of the model (section 5.5 to 5.7).

The outputs of the model include estimates of: i) the number of individual SE-positive eggs which caused illness; ii) number of illnesses; and iii) number of notified illnesses. Identifying the number of notified illnesses enables the model to determine: when an outbreak investigation is triggered; the length of time during which contaminated eggs continue to enter the market as the investigation progresses; and the number of illnesses not notified.

The base simulation model for the small and medium-sized farms in the first production cycle is run from the time the farm became SE-positive through to the end of the production cycle when the birds are 80 weeks old. Each SE-positive egg is tracked to determine if growth occurred, how the egg is used (as an egg meal or an ingredient), the setting where and how the egg is prepared and consumed (home or food service), and how many people become ill.

Health care-seeking behaviour is then modelled for each ill person.

In this section results for a single iteration of the model in the form of "Small Farm with refrigeration iteration #1". These results provide more detail as to how the different components of the model work together.

In this single iteration example, the model depicts a small farm producing eggs over a ten month period post-SE incursion (during the farm's first production cycle):

- The farm is SE-positive and hens are laying SE-positive eggs (24 weeks into the production cycle);
- Eggs from this farm have a refrigerated supply chain
- 0.1% of eggs laid are positive (221 of 220,172 eggs laid in the ten month period are SE-positive);
- Of the 221 positive eggs, 12 eggs (5% of SE-positive eggs, 0.005% of total eggs) cause illness. For each of the 12 eggs, the model considers:
  - Where the egg was prepared
  - How the egg was cooked
  - How many people consumed the egg
  - How many people became ill
  - The severity and duration of illness for each person
  - Whether the illness was notified (i.e. doctor seen, stool sample tested, strain typed)

This example for a small farm with refrigeration iteration #1 investigates the three scenarios above.

Explanation of model output: Small Farm with refrigeration iteration #1

A graphical summary of the model outputs for Small Farm with refrigeration iteration #1 is in Figure 10. Iteration #1 has a short supply chain (typical of smaller farms) and implements refrigeration for storage and transportation. The farm's environment became SE-positive five months into its first production cycle. The model then simulated a period of 42 days representing the time period dynamics of SE horizontal infection within the flock. Once this 42 days has elapsed, the flock as a whole is deemed SE-positive and the production of SE-positive eggs begins. The start of lay of SE-positive eggs is indicated by the vertical red line. The flock has 10-months left in its production cycle, after the production of SE-positive eggs begins, and in this time the 1,000 layer hens produced 220,172 eggs, of which 221 were SE-positive. Of these SE-positive eggs, 12 lead to cases of human illness. The remaining 209 SE-positive eggs didn't cause illness due to the inactivation of SE cells during preparation and/or the lack of SE growth in the egg prior to preparation. The relatively small number of illness-causing eggs is due to the low frequency of SE-positive eggs laid, the small supply chain length and through-chain refrigeration; eggs were kept cold for transportation and storage after grading, which inhibits SE growth inside the egg.

Panel (A) in Figure 10 and Figure 11 shows when these 12 SE-positive eggs were laid, prepared and caused one or more cases of illness. The black square in each row is the date of lay, the open circle is the date of preparation and the cross is the actual day notified cases are reported to the health department. The number at the right of each row is the number of actual cases predicted.

Figure 10 Panel (A) shows, in the absence of a PHS traceback system (i.e. no chance of farm identification or egg recalls) the total number of illness cases is 41. Of these 41 cases, only 6 would have been notified (indicated by the crosses). For instance, none of the six

illnesses from Egg #1 are notified; there are two notified cases for Egg #2; one notified case for Egg #7; one notified case for Egg #8, etc. Note Egg #2 was laid after Egg #1 but was prepared and consumed first (the length of the arrow in Figure 10 and Figure 11 represents time between lay and preparation). The number of actual illnesses versus those notified aligns with the expected underreporting factor of salmonellosis which is around 7 (Hall et al., 2006a, Hall et al., 2006b, Kirk et al., 2014).

Figure 10 Panel (B) shows the number of actual illness cases as they occur during the egg production cycle and Panel (C) shows the cases notified over the 10-month period. In this example, as Egg #2 resulted in two notified cases (from the seven total illnesses), this represents the definition of an outbreak (i.e. two or more cases directly linked). With PHS active, these notifications would result in an epidemiological investigation being triggered and ultimately the identification of the SE-positive farm.

Impact of environmental testing on Small Farm with refrigeration iteration #1

Figure 11 illustrates how the PHS rules for triggering an epidemiological investigation and identifying the farm (Panel (A)) were applied for the 12 eggs that resulted in illness from the Small Farm with refrigeration iteration #1 example. Figure 11 also shows how implementing on-farm environmental testing impacts how quickly an SE-positive farm can be identified (Panel (B) and Panel (C)).

The effect of PHS and traceback to the farm is shown in Figure 11 Panel (A). In this example, Egg #2 results in two illness notifications, thus representing an outbreak, which triggers an epidemiological investigation. Panel (A) shows the time to complete the investigation, farm confirmation testing and recall based on the two notified cases from the consumption of Egg #2 (indicated by the dashed line). At this point in the investigation, the farm is identified and supply of eggs is ceased. The time for an investigation can vary (e.g. SAGE members indicated between 3-60 days). The model uses a fixed amount of time required for epidemiological investigation, response and recall (e.g., 21 days for WGS clustering, 30 days for epidemiological investigations). During the investigation time, SE-positive Eggs #3 to #6 reach the market and are consumed. A total of 17 cases of illness are predicted until the farm is successfully identified and the eggs are recalled. PHS and the successful traceback to the farm prevent 24 illnesses from occurring, compared to Figure 10 (A) where 41 cases of illness would occur in the absence PHS.

Figure 11 Panel (B) shows the impact of implementing one on-farm environmental test per production cycle compared to PHS. For the one test per cycle scenario, the test is always performed at peak egg production, which is approximately nine weeks into a flock's laying cycle. In this example, there is no difference in the number of illnesses or the time taken to detect the SE-positive farm. The recall date for Panel (B) is the same as that for PHS in Panel (A). This is because the single environmental test undertaken for this example was completed before SE entered the layer environment. The farm would only be identified by PHS in the first production cycle; the single environmental test didn't reduce the illness burden. Further, if the investigation wasn't successful due to too few (or sporadically) notified cases, the farm would remain positive until the single environmental test of the second production cycle. This example highlights the risks with only implementing a single test system (further discussed below); i.e. where the burden of identifying the farm largely remains with the public health system, and requires a minimum of two illnesses from a single egg or three unrelated notified cases linked by genomic analysis, for an investigation to be triggered.

Figure 11 Panel (C) shows the impact of regular 13-week on-farm environmental testing. The recall date for this scenario is earlier than either PHS alone or PHS with a single

environmental test. Having additional tests during the farm's production cycle, allows the farm to be identified much earlier than relying on PHS. In this example, there will still be two notified cases, as the preparation date of Egg #2 is before the recall date and a total of 13 actual cases of illness will occur. However, the on-farm testing identifies the farm before any epidemiological investigation is completed and SE-positive Eggs #3 to #12 are prevented from entering the market and being prepared and consumed. Therefore, 28 illnesses do not occur (in the absence of a successful PHS; indicated in grey beyond the dashed line). Compared to PHS alone (which, in the model, always identifies the farm), the actual number of illnesses was reduced by 4, representing a 29% reduction in illness.

The number of days the farm was positive is calculated as the difference between the recall date and the date SE was introduced into the layer environment (Figure 11). This time is the same for PHS and PHS with a single environmental test. The time the farm is positive is reduced when regular 13 week testing is used.

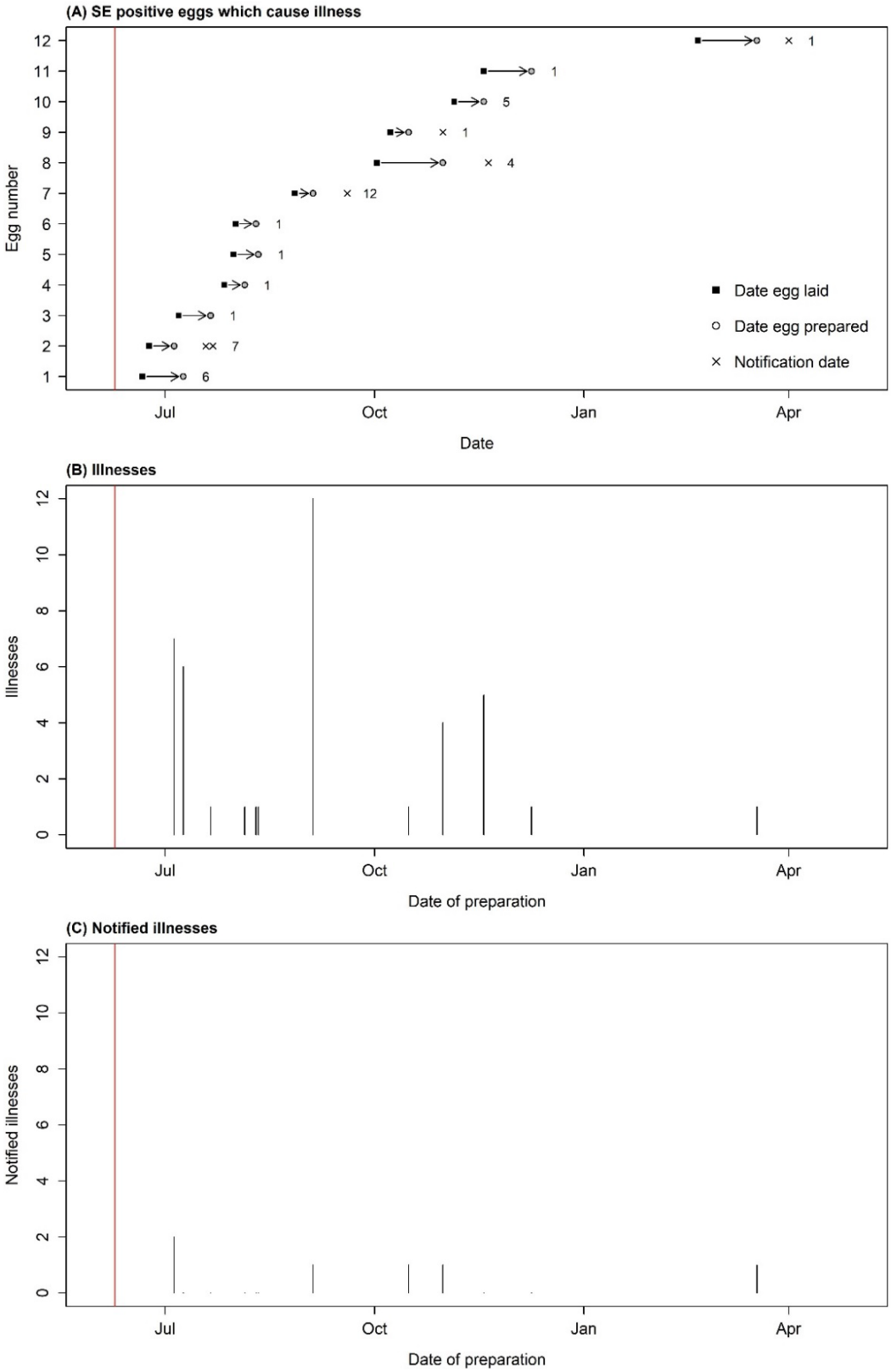

Figure 10: Small Farm with refrigeration iteration #1: (A) individual SE-positive eggs which caused illness, (B) number of illnesses and (C) number of notified illnesses.

The red solid line is the date the farm started producing SE-positive eggs. The black squares represent the date each egg was laid; the circles indicate the date the egg was prepared and consumed. The length of the arrow is the actual shelf-life of the egg; longer arrows indicate longer times. The crosses represent when notified cases were reported after visiting a doctor ad providing a stool sample, and the number indicates the total number of cases of illness caused by that egg.

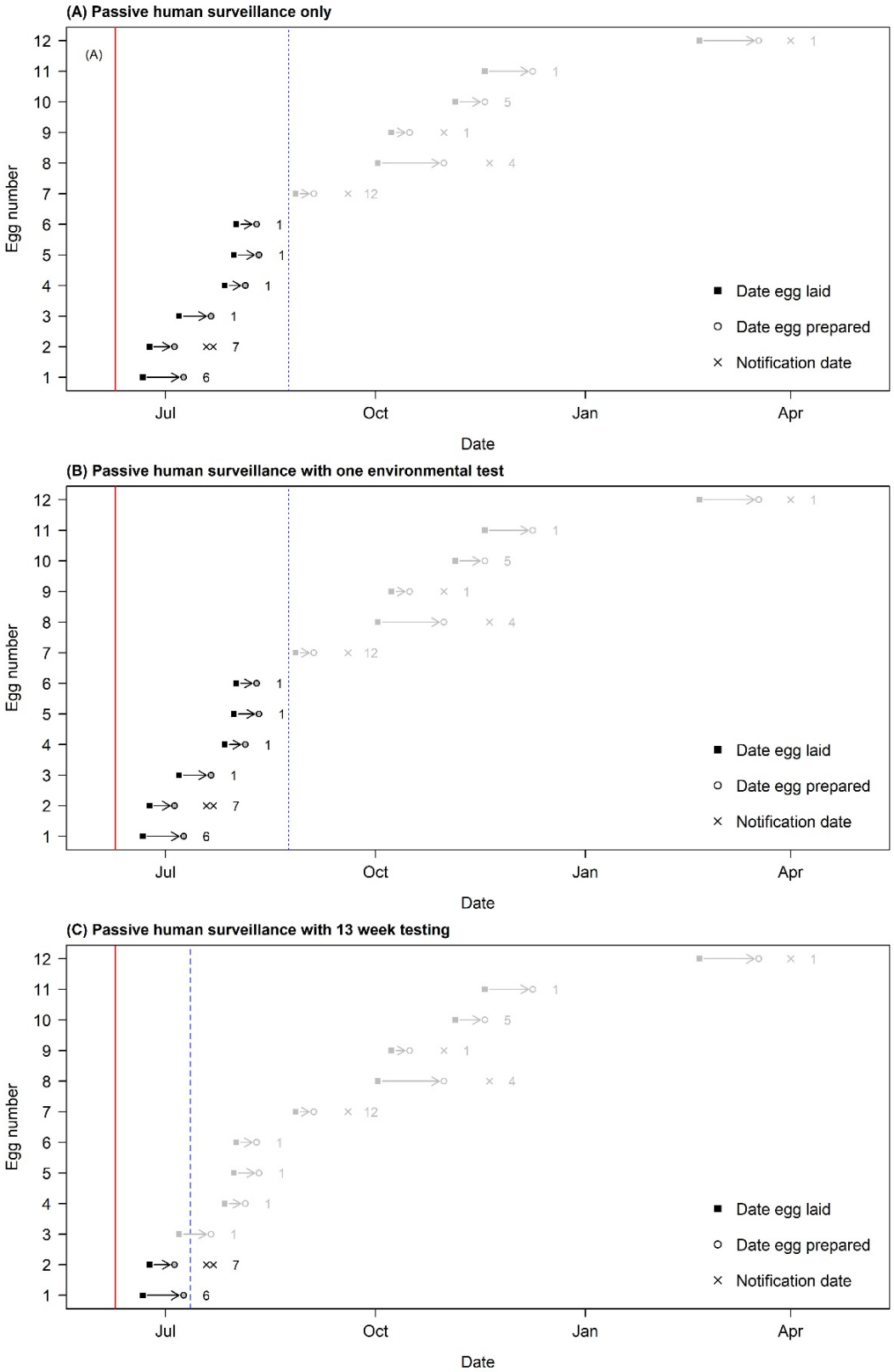

Figure 11: Small Farm with refrigeration iteration #1 – SE-positive eggs which caused illness with recall date: (A) PHS only, (B) PHS with one environmental test and (C) PHS with regular 13 week environmental tests. *See Figure 10 for explanation of interpretation the figure contents*

5.5 Combined analysis of environmental testing and temperature of supply chain

Section 5.4 presented the results of a single iteration (Small Farm with refrigeration iteration #1). It showed how the rules for identifying a farm using PHS or environmental testing are used. Each iteration uses different values for the date the farm becomes positive; the number of SE-positive eggs produced; temperature and time that an egg experiences through supply chain stages and the number of illnesses caused. By running the model many times a better understanding of the interactions between these factors can be obtained.

This section shows the output of modelling a positive farm over one production cycle and under four implemented scenarios: through-chain temperature control (ambient and refrigeration) and testing regimes (one test per cycle and regular 13-week tests). Each scenario is modelled for both a small and medium sized farm (20,000 hens). The model has been run 1000 times for each farm-scenario combination to enable analysis of the broad impact of the interventions on numbers of foodborne illness. The scenarios modelled are:

- one test per cycle and ambient supply chain
- one test per cycle and through-chain refrigeration
- regular 13-week tests and ambient supply chain
- regular 13-week tests and through-chain refrigeration

Using mean values (i.e. average of the number of illnesses) from the 1000 iterations provides insight into the impact of the mitigation strategies on number of illnesses. However, because of outliers, the mean does not show the distribution of illness numbers predicted. For this reason, median values, the 5^{th} and 95^{th} percentiles are presented for analysis. The median provides a better indication of what occurs most of the time on-farm.

The results presented below are for the 1,000 iterations. Summary tables for each figure are included in Annex 1. For the cost-benefit analysis, absolute numbers based on the mean outputs of the model have been generated and are discussed in section Annex 1.

5.5.1 Impact of temperature and environmental testing: medium size farm

Figure 12 shows the impact of different temperature supply chains (ambient or refrigerated) and different monitoring scenarios (PHS only, one test per cycle, regular 13-week tests) on number of illnesses from eggs from a positive medium size-farm.

The impact of the intervention measures (supply chain temperature and environmental testing) on number of illnesses under each scenario is similar for both small and medium-sized farms; only the medium size farm outputs are shown in Figure 12.

The results demonstrate implementing refrigeration through-chain decreases the total number of illnesses for all scenarios. A reduction in total illnesses is also seen when on-farm testing is implemented, with regular 13-week testing delivering the greatest impact.

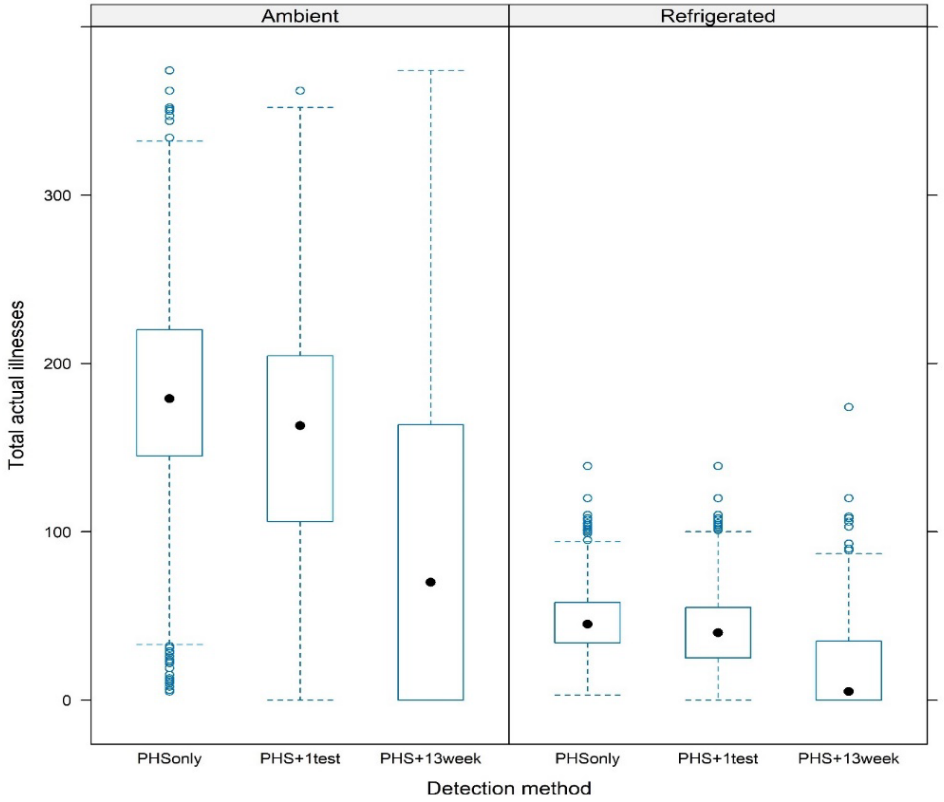

Figure 12: Box-and-whisker plot of predicted total cases of illness for a medium-sized farm by supply chain temperature and detection method: PHS only, PHS and 1 test per production cycle and PHS with regular 13 week tests.
The left panel shows eggs stored and transported at ambient temperatures. The right panel shows eggs stored and transported under refrigeration post grading. The black dots are the median values; the length of each box is the interquartile range with the distance between the 25th and 75th percentiles and represents the middle 50% of the data; the whiskers indicate the range of the data with individual points (open circles) being considered outlier values.

The cases of illness decrease when a single environmental test per production cycle is implemented on-farm, evident in both the ambient and refrigerated supply chain scenarios. This decrease is subtle and reflects that one test does not provide a huge benefit to reducing illness. When regular 13-week environmental tests are implemented, the number of illnesses decreases considerably for both supply chain scenarios.

The effect of refrigerating through the supply chain reduces the overall number of illnesses and has a tighter distribution over the 1,000 iterations; i.e. there is more confidence of consistently reducing illnesses when refrigeration is used. Figure 12 shows the distribution for total actual illnesses is large when there is an ambient supply chain. This is illustrated by the size of the box on the plot (representing the interquartile range of predicted values) and the length of the whiskers (representing the total range of values). This reflects that a 20,000 hen SE-positive farm can contribute a large number of contaminated eggs over time and with its longer supply chain, there is greater opportunity for SE growth inside the egg. This is consistent with the marked impact of refrigeration on reducing illnesses. A farm can have more confidence in preventing foodborne illnesses when through-chain refrigeration is in place.

The variability in the number of illnesses predicted by the model is vastly reduced with

refrigeration and occurs under all testing and PHS scenarios. Within an ambient supply chain, monitoring on-farm with regular 13-week testing can still be quite variable in reducing illness, but overall is more likely to reduce the likelihood of illness and remains a valuable tool within the management system. The variability in illness numbers with regular on-farm testing reflects the window between testing (13 weeks or a complete production cycle). This window causes inherent delay in detecting the farm has gone SE-positive and is producing contaminated eggs. Even with this variability, a farm with an ambient supply chain is more likely to detect SE on-farm before illnesses occur when applying regular 13-week testing.

Environmental monitoring: delivers an early intervention possibility

The effect of implementing on-farm environmental monitoring is best shown in Figure 13. This histogram shows the total number of illnesses for a medium-sized farm over 1,000 model iterations. The count (vertical axis) refers to the number of times the model predicts an illness. For both ambient and refrigerated supply chains, as testing is introduced, it is more likely the farm will be identified and have its contaminated eggs recalled prior to illnesses occurring. This likelihood increases with more frequent on-farm testing.

The number of 'zero illnesses' counted (vertical axis) increases with the single-test (2nd panel) and more so with the regular 13-week testing (3rd panel) when compared to PHS only (1st panel). This output supports the value of testing to detect SE on-farm shortly after it becomes present in the environment and before the flock has been infected and actively laying SE-positive eggs. On-farm environmental monitoring is a proactive public health and safety measure and does not rely on human illness for a farm to be identified as SE-positive.

Further, when refrigeration is applied, the impact is also a reduction in the severity of an outbreak: for instance, there are no counts of over 110 illnesses in any of the refrigerated scenarios.

The combination of through-chain refrigeration and regular 13-week testing provides a the greatest reduction in the number of human illness cases.

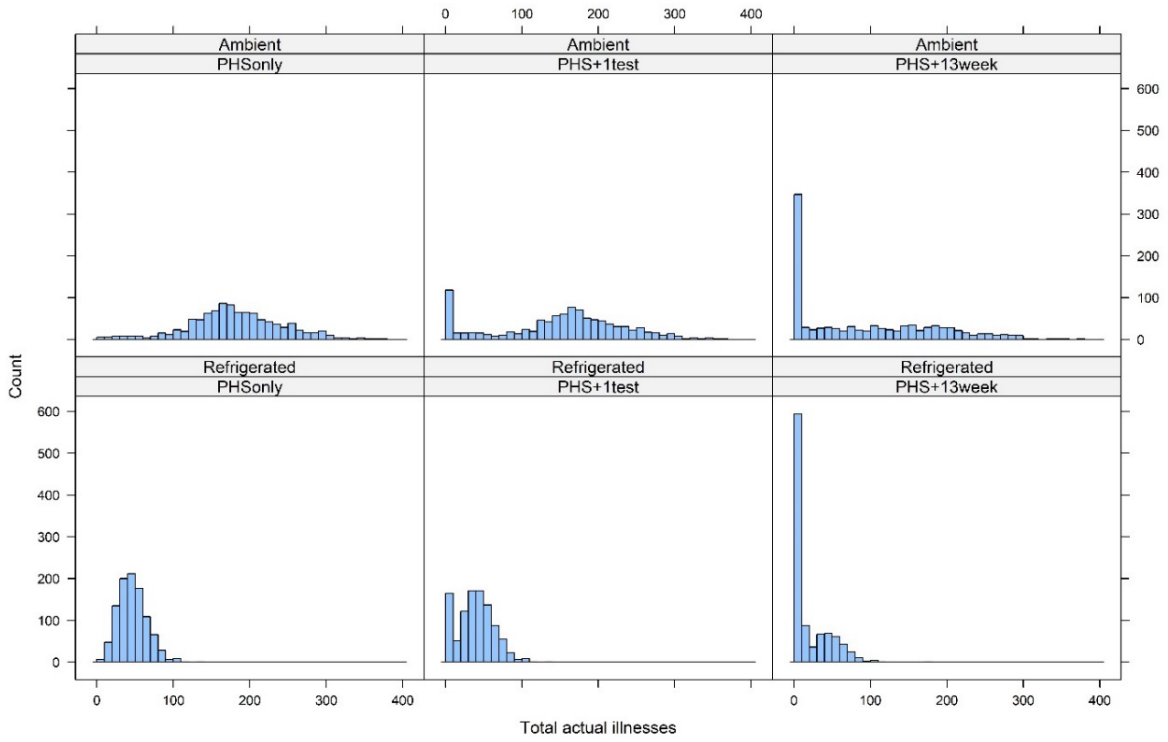

Figure 13: Histogram of total number of illnesses for a medium-sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS only, PHS and 1 test per production cycle and PHS with regular 13 week tests.
The top three panels are for eggs which are stored and transported at ambient temperatures. The bottom three panels are for eggs which were stored and transported under refrigeration.

5.5.3 *Impact of temperature and environmental testing: small farm*

The impact of environmental testing on-farm is similar for a small farm (Figure 14) as for a medium-sized farm. The trend of decreasing cases of illness for a single test per production cycle and regular 13-week testing is more pronounced for a small farm. This trend is especially noticeable for the refrigerated supply chain scenario.

Figure 14 also includes the distribution of total actual illness values for the model iterations where the small farm was not identified by PHS alone after three full production cycles (as discussed above; Figure 8). While the scale is much smaller than the illnesses associated with eggs from a medium size farm, a small farm with an ambient supply chain still causes illnesses. The cases of illness will continue to increase until the farm is identified, which may mean a large number of illnesses would have to occur for an outbreak to be declared.

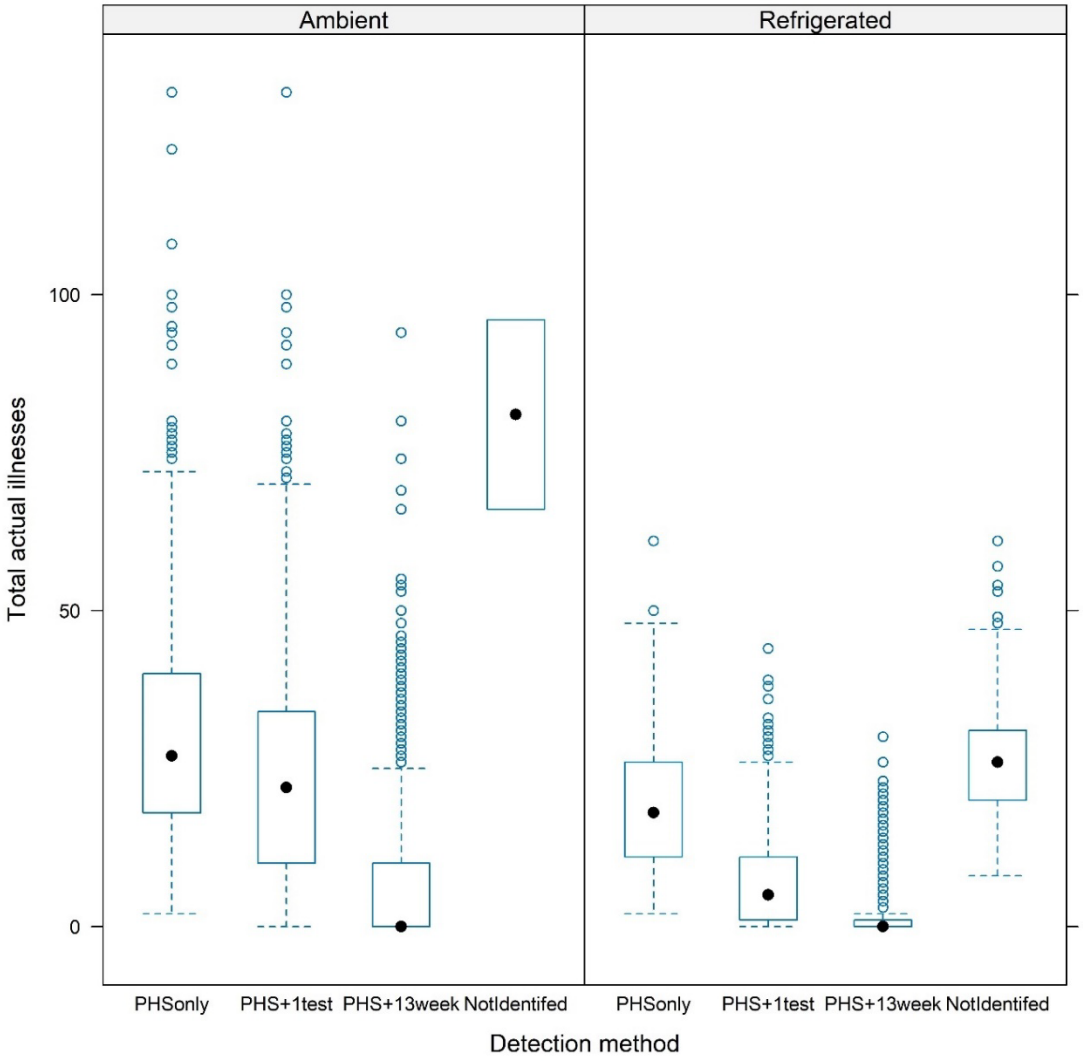

Figure 14: Box-and-whisker plot of predicted total cases of illness for a small farm by supply chain temperature and detection method: PHS only, PHS and 1 test per production cycle, PHS with regular 13 week tests, and not identified after three production cycles.
The left panel shows eggs after grading stored and transported at ambient temperatures. The right panel shows eggs stored and transported under refrigeration. The black dots are the median values; the length of each box is the interquartile range with the distance between the 25th and 75th percentiles and represents the middle 50% of the data; the whiskers indicate the range of the data with individual points (open circles) being considered outlier values.

Environmental monitoring: delivers an early intervention possibility

The results for environmental monitoring for a small farm are similar to if not more pronounced than the medium-sized farms and best shown in Figure 15. As was described above for medium-sized farms, for both ambient and refrigerated supply chains, as testing is introduced, it is more likely the farm will be identified prior to illnesses occurring. This likelihood increases with more frequent on-farm testing.

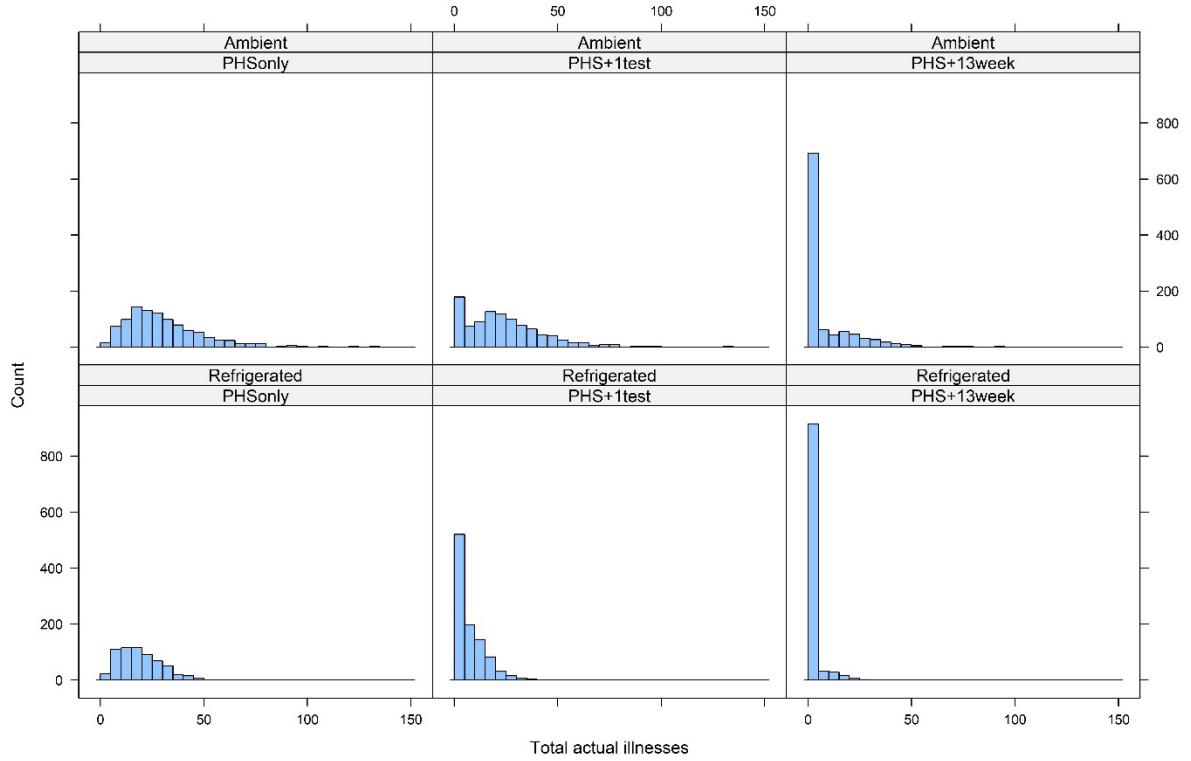

Figure 15: Histogram of total number of illnesses for a small-sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS only, PHS and 1 test per production cycle and PHS with regular 13 week tests.
The top three panels are for eggs which are stored and transported at ambient temperatures. The bottom three panels are for eggs which were stored and transported under refrigeration.

5.6 Illnesses avoided: impact of temperature and environmental testing

The model was used to investigate the number illnesses avoided when environmental testing is implemented compared to PHS alone; this is shown for small and medium-sized farms and for both one test per production cycle and regular 13-week testing. The model has been run for 1,000 iterations for each scenario.

For a medium-sized farm, the distribution of illnesses avoided values is shown in Figure 16. For each of the temperature scenarios, performing only a single test per production cycle may not lead to more illnesses avoided than relying on PHS alone. This is indicated on the graph by the large bar for the zero illnesses avoided value. This is because a single environmental test at peak production will only detect farms which were SE-positive prior to the single test.

For a typical 64-week production cycle (from hen age of 16- to 80-weeks) an environmental test around peak production (weeks 25) will only detect SE entry into the layer environment for up to around 10 weeks of the 64 week production cycle duration and only represents about 15% for this time. If SE enters the layer environment at week 26, the single environmental test would be too early and it would not be detected as being SE-positive until the farm's second production cycle test. This means 15% of the time, the single test avoids a large number of illnesses (as shown with the 5th percentile values), while 85% of time it shows no difference in illnesses compared to PHS (i.e. does not avoid any illness: illness avoided = 0).

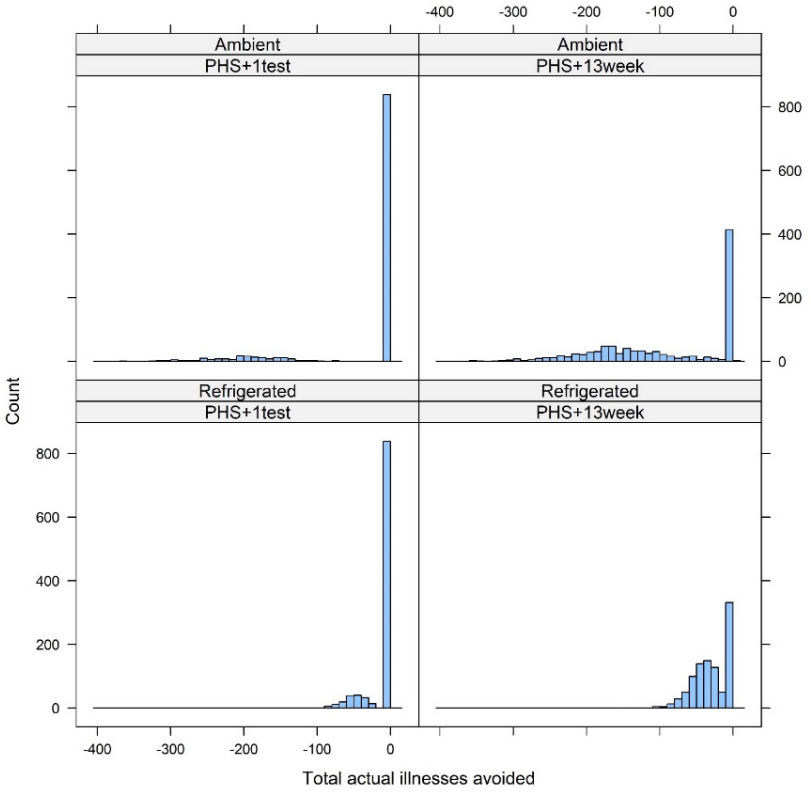

Figure 16: Histogram of illnesses avoided for a medium sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS and 1 test per production cycle or PHS with regular 13 week tests.

The top two panels are for eggs which are stored and transported at ambient temperatures. The bottom two panels are for eggs which were stored and transported under refrigeration. A value of zero (0) indicates that the testing regime did not reduce illness numbers compared to PHS. The more negative the illnesses avoided value is, the greater impact the testing regime had on reducing illness.

By contrast, regular 13-week environmental tests, on a medium-sized farm, are more likely to detect an SE-positive farm before PHS alone and thus lead to more illnesses avoided. This trend is shown in Figure 16, where for one test the majority of illnesses avoided values over 1,000 iterations are stacked on zero illnesses avoided. When 13-week testing is applied the histogram bar representing zero illnesses avoided decreases in size. Regular 13-week testing shows a wide distribution of illnesses avoided values, which illustrates a higher likelihood of avoiding illness with this testing strategy.

The absolute reduction in number of illnesses alone does not provide the full picture for the effectiveness of environmental testing. The actual number of illnesses avoided needs to be considered in context of how many illnesses occur with PHS only. The percent reduction helps illuminate the true reduction. Figure 17 depicts the percentage reduction of illnesses for a medium-sized farm. In this figure a value of -100% indicates that all of the predicted cases of illness would be avoided with environmental testing. For each of the temperature scenarios where only a single environmental test at peak production is made, the testing is more likely to contribute a 0% reduction in illness. For a regular 13-week environmental testing regime the percentage reductions show a bimodal distribution where 0% or 100% reduction in illness are similarly likely. When refrigeration through-chain is applied the likelihood of a 100% reduction increases.

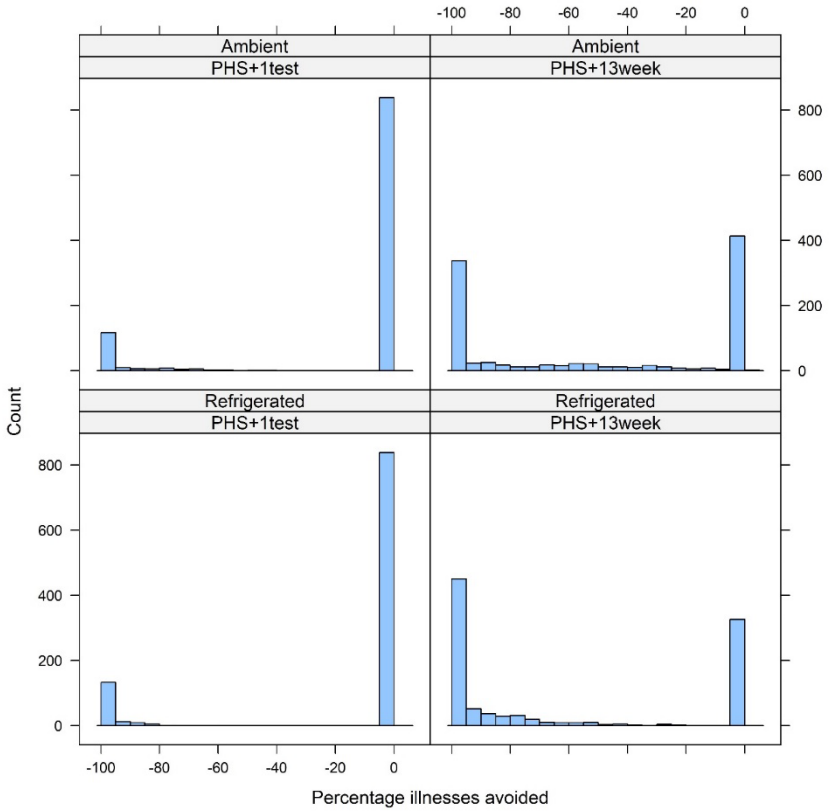

Figure 17: Histogram of percent reduction for the number of illnesses avoided on a medium sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS and 1 test per production cycle or PHS with regular 13 week tests.

The top two panels are for eggs which are stored and transported at ambient temperatures. The bottom two panel are for eggs which were stored and transported under refrigeration. A value of zero (0) indicates the testing regime did not reduce illness numbers compared to PHS. A value of -100 indicates 100% of the illnesses in a PHS only system were avoided by introduction of the testing regime.

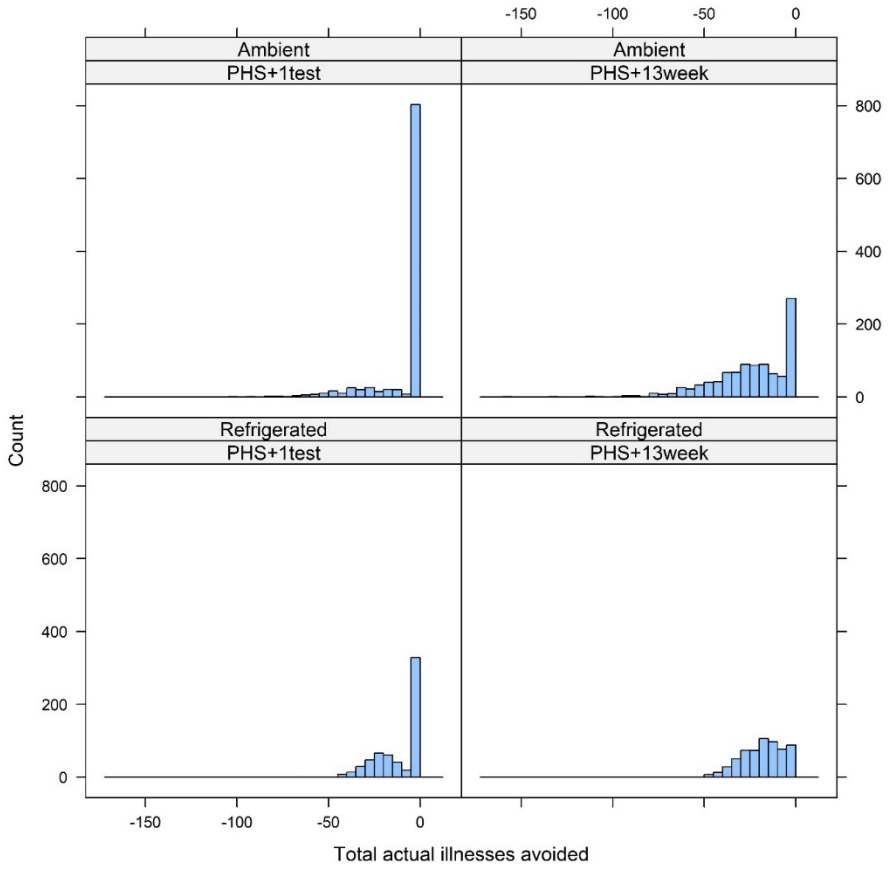

Figure 18: Histogram of illnesses avoided for a small sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS and 1 test per production cycle or PHS with regular 13 week tests.

The top two panels are for eggs which are stored and transported at ambient temperatures. The bottom two panels are for eggs which were stored and transported under refrigeration. A value of zero (0) indicates that the testing regime did not reduce illness numbers compared to PHS. The more negative the illnesses avoided value is, the greater impact the testing regime had on reducing illness.

For a small-sized farm, the distribution of illnesses avoided values over 1,000 iterations of the model is shown in Figure 18. Similar to the results for a medium-sized farm once per cycle testing is much more likely to not avoid any illnesses. For a small farm with a refrigerated supply chain this observation is not as prominent. Regular 13-week environmental tests implemented on a small farm results in a similar trend of illnesses avoided when compared to medium farms. SE-positive farms are more likely to be detected by 13-week testing before PHS alone and therefore illnesses are likely to be avoided. The trend again is more pronounced for a small farm when compared to a medium farm.

Figure 19 depicts the percentage reduction of illnesses for a small-sized farm. In this figure a value of -100% indicates that all of the predicted cases of illness would be avoided with environmental testing. Similar to a medium-sized farm, for each of the temperature scenarios where only a single environmental test at peak production is made, the testing is more likely to contribute a 0% reduction in illness. For a regular 13-week environmental testing regime the percentage reductions show a much higher likelihood of a 100% reduction in illness for a small-sized farm. This is a more pronounced trend then for medium sized farms. When refrigeration through-chain is applied in combination with 13-week testing the likelihood of a 100% reduction increases further. Note that in Figure 19, there are not 1,000 iterations

graphed for the small farm refrigerated supply chain scenarios. This is because, as described previously, there is only a 62% chance of PHS alone identifying a small farm under these conditions after three cycles when the model was stopped. Therefore, in reality testing of either frequency is more effective at reducing the burden of illness than illustrated here.

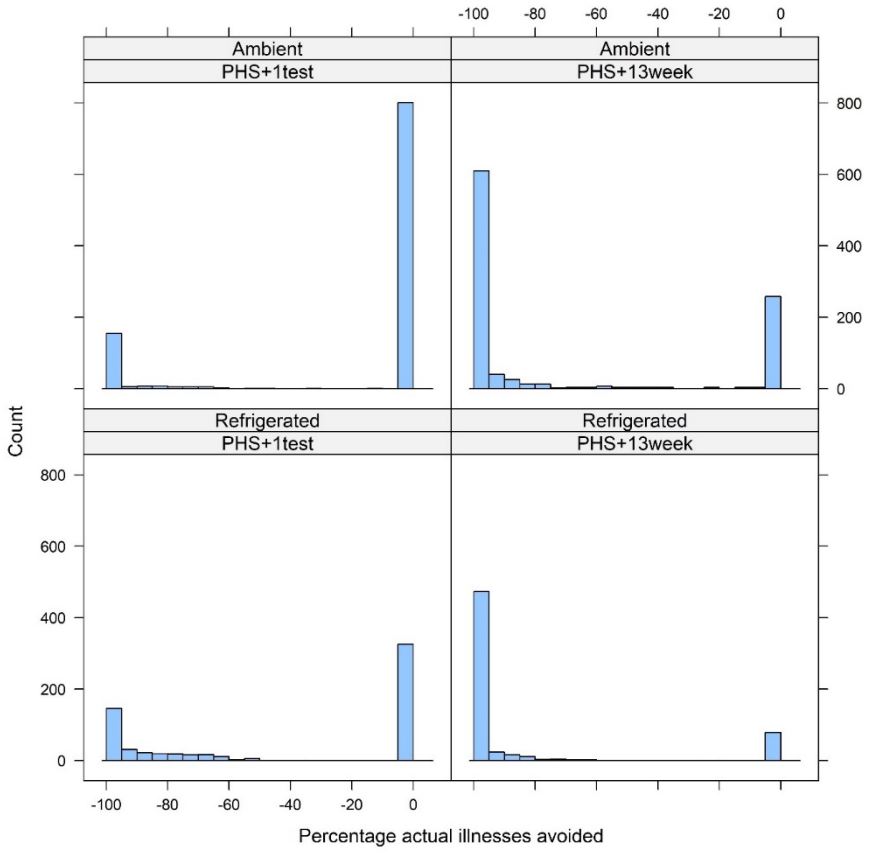

Figure 19: Histogram of percent reduction for the number of illnesses avoided on a small sized farm over 1,000 model iterations by supply chain temperature and detection method: PHS and 1 test per production cycle or PHS with regular 13 week tests.
The top two panels are for eggs which are stored and transported at ambient temperatures. The bottom two panel are for eggs which were stored and transported under refrigeration. A value of zero (0) indicates the testing regime did not reduce illness numbers compared to PHS. A value of -100 indicates 100% of the illnesses in a PHS only system

5.7 Duration a farm is SE-positive

The model determined the number of days a farm is SE-positive before it is identified (medium farm, Figure 20; small farm, Figure 21; 1000 iterations).

When regular 13-week environmental tests are applied, the time before the farm is detected is similar regardless of temperature of the supply chain. Regular testing decreases the total time the farm is SE-positive compared to a single test and PHS only. This trend is shared for both ambient and refrigerated supply chains, i.e. regular testing, regardless of supply chain temperature allows for early detection of a SE-positive farm.

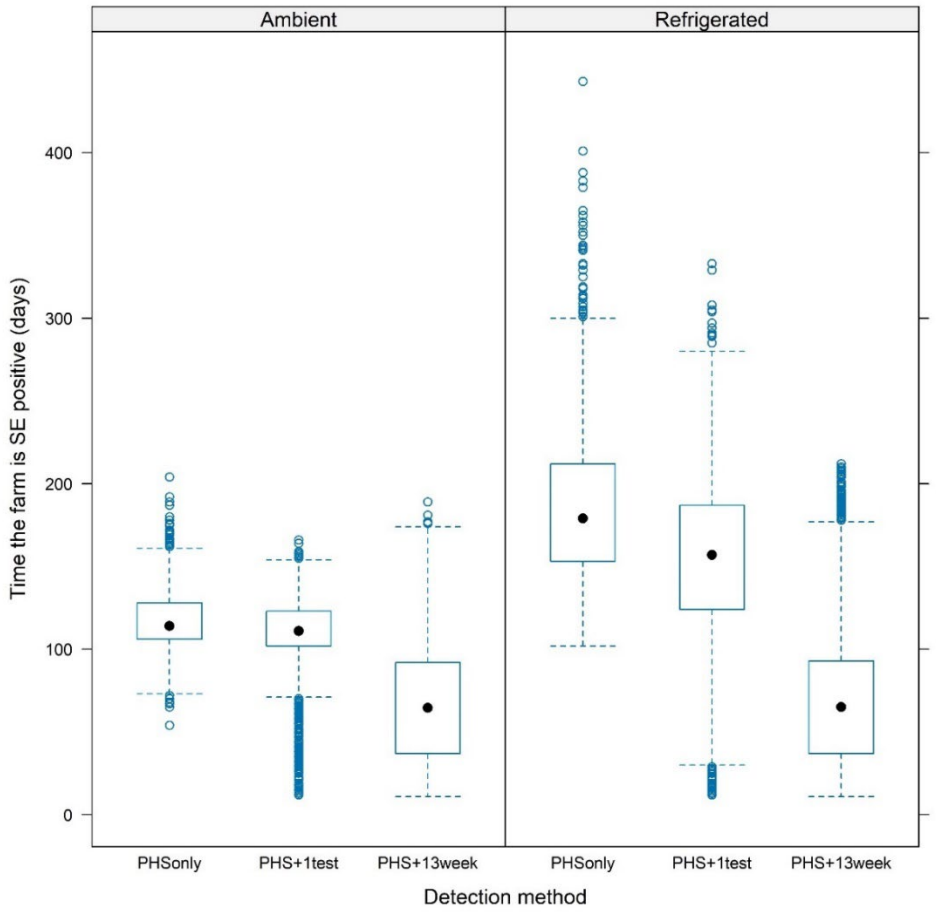

Figure 20: Box-and-whisker plot of the amount of time a medium-sized farm is SE-positive before identified by PHS or detection method (PHS only, PHS with one test per production cycle and PHS with regular 13 week tests).
The left panel are for eggs which are stored and transported at ambient temperatures. The right panel are for eggs which were stored and transported under refrigeration. The black dots are the median values; the length of each box is the interquartile range with the distance between the 25th and 75th percentiles and represents the middle 50% of the data; the whiskers indicate the range of the data with individual points (open circles) being considered outlier values.

In small farms (Figure 21), the pattern observed in medium-sized farms is more evident. Implementing on-farm testing shortens the SE-positive period, with the 13-week test being the most effective in early detection. The small farm with a refrigerated supply chain demonstrates increased variability in the amount of time the farm is SE-positive before detection when only PHS is active. This relates to scale: fewer eggs, fewer contaminated eggs, fewer cases of illness makes linking cases difficult for public health departments. Figure 21 has an additional category not included in Figure 20 for medium farms; small farms not identified by PHS after the completion of three production cycles. Figure 8 (also refer to the Table for Figure 8 in Annex 1) shows that where PHS only is operating 0.2% and 38.4% of farm iterations with ambient and refrigerated supply-chains would not be detected by the end of the third production cycle. As a result these farms will have the longest time being SE-positive, and would continue to accrue additional time in to the fourth and subsequent production cycles.

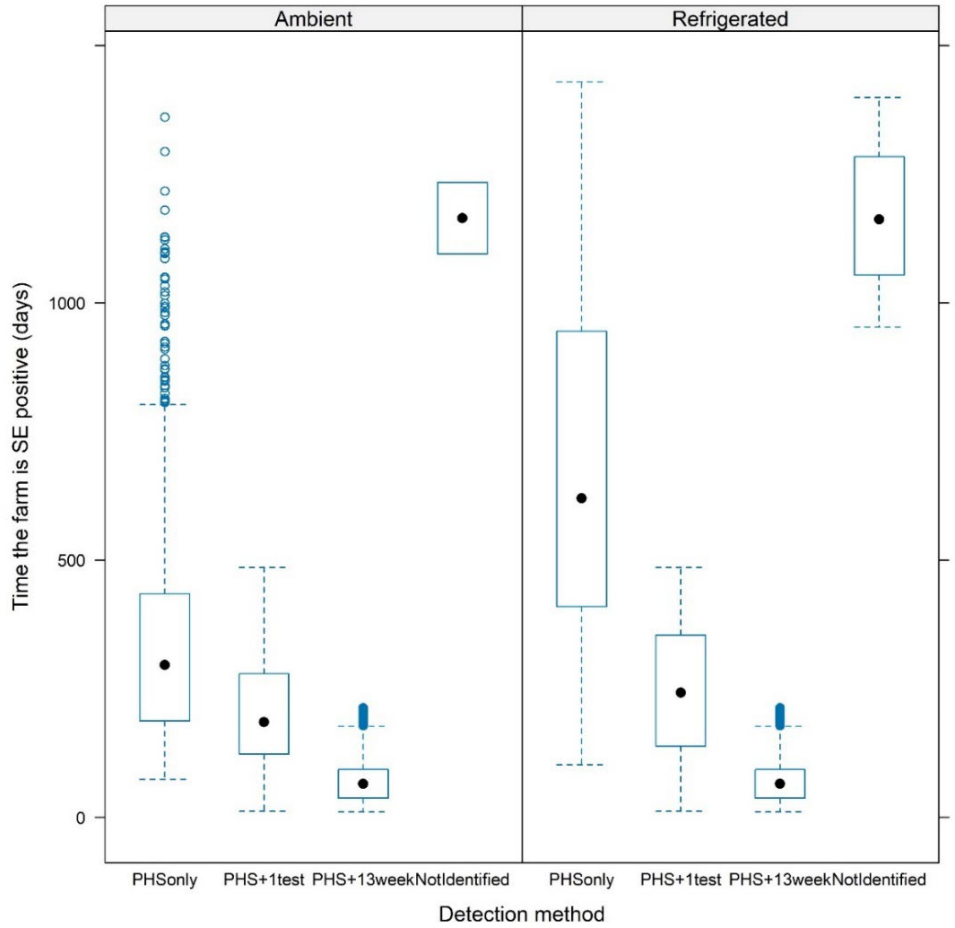

Figure 21: Box-and-whisker plot of the amount of time a small-sized farm is SE-positive by detection method (PHS only, PHS with one test per production cycle and PHS with regular 13 week tests) after three complete production cycles.
The left panel are for eggs which are stored and transported at ambient temperatures. The right panel are for eggs which were stored and transported under refrigeration. The black dots are the median values; the length of each box is the interquartile range with the distance between the 25th and 75th percentiles and represents the middle 50% of the data; the whiskers indicate the range of the data with individual points (open circles) being considered outlier values.

5.8 Conclusion

Refrigeration of eggs from both small and medium size farms, through the supply chain greatly decreases human illness. Refrigeration prevents growth of many microorganisms including SE if present in egg contents. A farm can have more confidence in preventing foodborne illnesses when through-chain refrigeration is in place.

The total number of illnesses is much lower for a small farm: this relates to scale (fewer eggs produced) and also that its supply chain is generally shorter, decreasing the time available for SE growth. Longer supply chains provide greater opportunity for SE growth inside the egg, if present. This also explains the marked impact of refrigeration on reducing illnesses; refrigerating the supply chain for a medium-sized farm has greater impact than for a small farm because its supply chain is typically longer.

Environmental testing on-farm contributes to decreasing the overall burden of illness and also decreases the total time a farm remains SE-positive. Increasing the frequency of testing

to every 13 weeks produces a pronounced decrease in illness. A single test during a flock's production cycle is not as efficient as regular 13-week testing at protecting public health, and may not lead to more illnesses avoided than relying on PHS.

When implemented in tandem, 13-week environmental monitoring and refrigeration result in the greatest reduction in illness.

Implementing a testing regime also reduces the amount of time a farm will remain SE-positive, irrespective of size. This reduction is greatest when regular 13-week testing is performed. Minimising the duration a farm is SE-positive leads to fewer SE-contaminated eggs entering the market and thus lowers the number of illnesses caused. Moreover, this reduction helps eliminate the possibility that a farm becomes a SE-reservoir that can spread SE to neighbouring properties or production facilities.

6 DATA GAPS

Uncertainties and data gaps are common in risk assessment, especially with microbiological hazards. Best practice supports the use of models and informed values to further the risk assessment.

Data gaps were identified throughout the egg and egg product supply chain contributing to uncertainty in risk assessment outputs. Gaps include Australian data on prevalence and levels of *Salmonella* contamination in and on eggs, transmission through the shell, effects of hen age, and vertical transmission of non-SE salmonellae.

Conservative assumptions, based on literature, expert opinion, experiences in other countries, have been incorporated into the model to account for uncertainty and these are identified for transparency (refer to SD2). Further research to reduce uncertainty is discussed in Annex 2.

7 RESPONSES TO RISK ASSESSMENT QUESTIONS

1. How has the food safety risk changed for eggs since the 2012 Proposal P301 risk assessment?

- SE internally contaminated eggs (i.e. vertically transmitted) have been detected in Australian egg layer flocks and were associated with multiple salmonellosis outbreaks and cases, including the major 2018-19 incident.
- SE strains endemic to Australia have been identified.

The P301 risk assessment underpinning Standard 4.2.5 mainly focused on the risk associated with horizontal transmission of *Salmonella* spp. into the egg and egg content. The assessment was based on eggs becoming food safety risks from hazards being introduced via cross contamination, during production or processing of eggs. The assessment did not consider the potential of internal contamination of eggs and its growth, or inactivation of the pathogen with time and temperature.

Food safety risks with Australian table eggs changed when SE was detected in the Australian egg production system in 2018–2019. Although prevalence of SE in Australian laying flocks is limited and sporadic, outbreaks from consuming SE-contaminated eggs have been reported in Queensland and NSW in 2023 (unpublished). When evaluating SE from 98 countries including Australia, Luo et al. (2023) divided Australian SE strains into three phylogenetic clades (A, B and C). Clades A and C represented 16.4% and 3.5% of the total isolates, respectively, and were of local origin. This result demonstrates the presence of SE strains endemic to Australia, which can, and are likely to continue to, sporadically occur in layer flocks.

2. What on-farm practices, risk factors and controls would address the new food safety risks for eggs?

- The practices, risk factors and controls currently in place in Australia, whether voluntary or regulatory, vary across different egg farming systems and size of farms. This means SE contamination pathways and management also varies widely.
- A combination of strategies must be used to control SE risks; it must be a whole of system approach. Strategies include biosecurity measures, vaccination, animal and pest control, hygiene, environmental monitoring and egg temperature control.

There are multiple intervention strategies used internationally to control SE in table eggs, all of which involve systematic control of risks. Some countries also prescribe either a (short) shelf life or refrigeration as a means to reduce growth of SE, if potentially present. Farm management programs using integrated interventions at multiple stages of egg production and distribution are needed to prevent SE contamination of eggs and protect public health. Tools that prevent introduction of SE into an egg-production facility include biosecurity, procurement of SE-free replacement flocks, and keeping disease vectors (i.e. animals and pests) out of houses. Effective cleaning of chicken houses between flocks and applying effective disinfectants reduces environmental contamination and the likelihood of transmitting SE to successive flocks. Vaccines can be used to increase the resistance of layer hens to intestinal colonisation by SE, systemic infection, and production of contaminated eggs. Environmental testing can pinpoint potential sources of SE contamination and early. *Salmonella* can survive in various environments, including farms, processing facilities, equipment, and even water sources. Regular testing in these areas can detect presence of

Salmonella and trigger corrective actions to prevent further spread. Lowering the internal temperature of fresh eggs to 7°C or less as soon as possible after laying and maintaining this temperature during transport and storage will prevent SE multiplication in potentially contaminated eggs (see section below).

The above proactive measures help break the cycle of infection and lower the chances of contaminated products reaching consumers, thereby reducing incidence of foodborne illness.

3. For supply chain management, when do eggs become potentially hazardous and how would this be managed?

- Similar to when egg contents become contaminated from leaking eggs or surface contamination, when a foodborne pathogen is inside an egg, that egg supports microbial growth. Because SE can be vertically transmitted during egg formation, it poses a higher risk than other pathogens.
- No single time or stage of production and processing can be identified for when an egg has become a potentially hazardous food; it depends on the management system through chain.
- Because of the complexity and variability of production and supply chains, multiple measures provide the greatest confidence in preventing contaminated eggs from causing human illness.
- As current requirements do not protect from vertically transmitted contamination, additional measures including temperature control, on-farm monitoring and enhancing biosecurity on farm can improve human health outcomes. Further, enhancing traceability allows faster identification of a positive farm and stopping further supply of contaminated eggs.
- Refrigeration is required to prevent growth of SE when eggs are internally contaminated with SE.

SE is not considered to be endemic in Australian laying flocks. However, the frequency of *Salmonella* outbreaks linked to consuming SE-contaminated eggs suggests there is undetected SE in Australia; sporadically within Australian commercial laying flocks, the environment and/or wild birds. The 2018-2019 outbreak and subsequent continued sporadic occurrences of SE (i.e. detections on farm and human illnesses) indicates current requirements do not adequately manage risks.

Temperature control can inhibit the potential growth of SE within an egg. It helps to maintain the integrity of the yolk membrane and maximise the time before exponential SE growth can occur. Storage at 4°C is reported to preserve the antimicrobial agents of the albumen and maintain the integrity of vitelline membranes. *Salmonella* contamination in and on eggs is influenced by the eggs' storage/transport temperature. The effectiveness of refrigeration on preventing SE growth depends on several factors including the initial level and location of contamination during egg formation (i.e. inside or outside the egg).

The risk of SE egg contamination is low because the prevalence of SE in Australia is currently low. However, there are SE strains endemic in Australia and internal egg contamination with SE has been found. Therefore, there is potential for SE to be found within a commercially farmed egg and for subsequent SE growth within the egg, meaning eggs become a potentially hazardous food.

Environmental monitoring along with good biosecurity practices must work in tandem with refrigeration to effectively prevent human illness from SE contaminated eggs.

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9 ANNEXES

ANNEX 1: Risk characterisation summary tables for figures

Table for Figure 8: Cumulative percentage of medium and small farms identified by PHS only by production cycle

Production cycles	Medium farm with ambient supply-chain	Medium farm with refrigerated supply-chain	Small farm with ambient supply-chain	Small farm with refrigerated supply-chain
0	0	0	0	0
1	89.2	78.4	49	10.6
2	100	100	96.3	40.3
3	100	100	99.8	61.6

Table for Figure 9 Cumulative percentage of small farms with refrigerated supply-chains identified by production cycle

Production cycle	PHS with regular 13 week environmental tests	PHS a single environmental test per cycle	PHS only
0	0	0	0
1	81.5	16.2	10.6
2	100	100	40.3
3	100	100	61.6

Table for Figure 10 and Figure 11

Egg number	Date of lay[†]	Date of preparation	Date of notification	Total actual illnesses
1	21/06/2000	9/07/2000	NA	6
2	24/06/2000	5/07/2000	19/07/2000 & 22/07/2000	7
3	7/07/2000	21/07/2000	NA	1
4	27/07/2000	5/08/2000	NA	1
5	31/07/2000	11/08/2000	NA	1
6	1/08/2000	10/08/2000	NA	1
7	27/08/2000	4/09/2000	19/09/2000	12
8	2/10/2000	31/10/2000	20/11/2000	4
9	8/10/2000	16/10/2000	31/10/2000	1
10	5/11/2000	18/11/2000	NA	5
11	18/11/2000	9/12/2000	NA	1
12	20/02/2001	18/03/2001	1/04/2001	1

[†]The model start date was set as 1 January 2000 when 16 week old pullets start production cycle 1.

Table for Figure 12 to Figure 15: Total number of illnesses: median (5th, 95th percentiles) – baseline, testing and temperature[1]

Farm size	Temperature scenario	PHS only	PHS + 1 test/cycle	PHS + 13 week tests	Not identified
Small	Ambient	27 (9, 64)	22 (0, 59)	0 (0, 35)	81 (-, -)
Small	Refrigeration	18 (6, -)*	5 (0, 22)	0 (0, 11)	26 (14, 43)
Medium	Ambient	179 (78, 287)	163 (0, 277)	70 (0, 257)	None
Medium	Refrigeration	45 (20, 80)	40 (0, 78)	5 (0, 68)	None

[1]PHS only; PHS + 1 environmental test per cycle; PHS with regular 13 weeks tests; by farm size and temperature scenario, median (5th, 95th percentiles)

* 95th percentile not reported as 384 of 1,000 iterations the farm was not identified by PHS by the end of production cycle 3 (see Figure 8); too few notified cases were predicted to trigger the requirements for an epidemiological investigation.

Table for Figure 16 and Figure 18: Number of illnesses avoided, median (5th, 95th percentile)

Farm size	Temperature scenario	PHS with one test/cycle	PHS with 13 week tests
Small	Ambient	0 (-44, 0)	-21 (-62, 0)
Small	Refrigeration	0 (-33, -)*	-17 (-37, -)*
Medium	Ambient	0 (-218, 0)	-88 (-245, 0)
Medium	Refrigeration	0 (-56, 0)	-29 (-70, 0)

* 95th percentile not reported as 384 of 1,000 farms were not identified by PHS by the end of production cycle 3 (see Figure 6A); too few notified cases were predicted to trigger the requirements for an epidemiological investigation.

Table for Figure 17 and Figure 19: Percent reduction of the number of illnesses avoided, median (5th, 95th percentile)

Farm size	Temperature scenario	PHS with one test/cycle	PHS with 13 week tests
Small	Ambient	0 (-100, 0)	-100 (-100, 0)
Small	Refrigeration	0 (-100, -)*	-100 (-100, -)*
Medium	Ambient	0 (-100, 0)	-50 (-100, 0)
Medium	Refrigeration	0 (-100, 0)	-90 (-100, 0)

* 95th percentile not reported as 384 of 1,000 farms were not identified by PHS by the end of production cycle 3 (see Figure 6A); too few notified cases were predicted to trigger the requirements for an epidemiological investigation.

Table for Figure 20 and Figure 21: Number of days that a farm is SE-positive, median (5th, 95th percentiles)

Farm size	Temperature scenario	PHS only	PHS + 1 test/cycle	PHS + 13 week tests
Small	Ambient	296 (120, 736)	185 (34, 402)	65 (16, 182)
Small	Refrigeration	620 (188, -)*	242 (34, 456)	65 (16, 188)
Medium	Ambient	114 (99, 286)	111 (34, 145)	64 (16, 148)
Medium	Refrigeration	179 (122, 292)	157 (34, 236)	65 (16, 179)

* 95th percentile not reported as 384 of 1,000 farms were not identified by PHS by the end of production cycle 3 (see Figure 6A); too few notified cases were predicted to trigger the requirements for an epidemiological investigation. For those farms which were not identified by the end of production cycle 3 (Figure 6A) the total time that they were SE-positive was 714 (522, 933) days. The predicted number of cases of illness for these undetected farms was 26 (14, 43). The median number of cases for detected farm was 16 cases.

ANNEX 2: Data gaps

Prevalence of *Salmonella* contaminated eggs (external and internal contamination)
With the low prevalence of *Salmonella* contaminated eggs in Australia, a previous large survey (i.e. 20,000 eggs) did not detect *Salmonella* either on the egg surface or in egg contents (Daughtry et al., 2005). The size of a survey necessary to determine the prevalence of contaminated eggs with statistical confidence would be extremely costly. However, using in the quantitative model estimates of prevalence based on reported frequency of egg contamination in SE positive flocks, the relative risk from changes in the rate of *Salmonella*-contaminated eggs could be determined.

Hen physical state that affect SE-positive egg production
It is unclear if and how hen age affects production of SE-positive eggs including if they increase with hen age? Literature from the USA shows moulting has an effect on SE egg prevalence. It is unclear how common moulting is in Australian commercial laying farms.

Levels of *Salmonella* in contaminated eggs
Very few studies have determined the initial level of *Salmonella* in contaminated eggs at, or near, the point at lay. Available data on the level of *Salmonella* in contaminated eggs has been generated from experimentally infected laying hens, which may not be representative of eggs from naturally infected hens. Results from the quantitative model estimate the risk of illness from consuming raw eggs that have been stored and eaten prior to the opportunity for growth of *Salmonella* depends on the initial number of organisms in the egg contents. A survey on *Salmonella* levels in contaminated eggs would need to consider variables such as the breed, age and health status of hens, and the serotype and strain of *Salmonella*.

Vertical transmission of non-SE *Salmonella* serovars
For non-SE *Salmonella* serovars, horizontal transmission is considered the main route of egg contamination (i.e. from dirty or broken eggs). Studies have shown some non-SE *Salmonella* serovars can colonise the reproductive tissue of hens under experimental conditions. Equivalent studies to determine the possibility/extent of vertical transmission using *Salmonella* serovars isolated from Australian laying flocks may validate assumptions made in the risk assessment.

Mechanisms and extent of horizontal transmission of *Salmonella* into egg contents
Many factors are associated with the potential transmission of *Salmonella* through the egg shell (and membranes) into the egg contents. Studies could investigate contaminated eggs from naturally infected hens and penetration of *Salmonella* through the shell and under conditions observed during production and processing of eggs in Australia.

Mechanism of *Salmonella* growth within an egg and update to YMT model
The original YMT model was based on inoculation of egg albumen with high levels (500 CFU) of SE. Based on results from international studies, some non-SE *Salmonella* serotypes are able to internalise and survive in egg albumen. However, data is insufficient to assess how storage temperatures affect the ability of non-SE serotypes to internalise eggs, survive in albumen, and/or grow in egg yolk.

How epidemiologists and public health units investigate an outbreak
Foodborne illness data collected during outbreak investigations can support a strong feedback mechanism to prevent food contamination through effective risk mitigation measures. Information gathered during interviews of cases, how that data is used to connect cases and declare an outbreak, traceback methodology including if common across jurisdictions is valuable information at identifying where and when in a food production chain, a food has become a risk. Future microbiological risk assessments would also greatly benefit

from access to deidentified data from OzFoodNet's hypothesis-generating questionnaire.

How national foodborne illness databases are maintained
NNDSS and WGS facilities generate and source information that is critical to identifying pathogen strain which is essential in understanding the movement of pathogens through the food supply chain, from production to consumption. Information on how numbers are generated in the NNDSS would support model assumptions (i.e. PCR, PCR + Culture, Culture only?)

Specific MLVAs and their association with eggs
For isolates that have MLVAs, the risk assessment would benefit from source attribution data.

Prevalence of use for egg chain pathways (from lay to consumption)
Detailed information on each egg supply chain pathway, egg preparation and food type in Australia would support the model. Currently this information is not collected in the Australian Total Diet Study.

Supply chain information
Data on length of supply chains, egg storage at retail (supermarkets, fruit and vegetable stores, butchers, delis etc) including percentage of eggs sold at ambient or refrigeration would validate information used in the model.